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THE DIAPHRAGM AND DIAPHRAGMATIC HERNIA

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I have divided this paper into two parts, the first dealing in detail with the development of the diaphragm, of which there is, to my mind, no adequate description, and the second attempting to correlate this development with the various kinds of diaphragmatic hernia observed by others, as gathered from the literature. For readers chiefly concerned with the clinical aspects of the subject the second part may suffice; those who like to trace the embryologic causes of anomalies may find the first part of interest.

I

The diaphragm is composed of the tendinous centrum and the diaphragmatic muscles, the two portions arising from different sources. The centrum originates as the septum transversum, so named by His[1] in 1880. In a human embryo of about 3 weeks (fig. 1) this structure is a transverse plate or shelf of mesoderm extending from the ventral body wall to the ventral wall of the foregut, that part of the intestinal tract which is carried forward in the general growth of the head. It forms the caudal wall of the pericardial cavity and is pierced dorsally by the fused ends of the vitelline veins, which arise in the yolk sac and follow the yolk stalk to the caudal end of the heart. The plane in which the septum lies slants ventrally downward, its angle varying with the varying growth of the foregut and the thoracic wall. The ventral attachment continues half way around the side walls to meet the umbilical veins, which, coming from the placenta in the umbilical cord, course in the lateral wall to enter the heart by passing through the dosal edge of the septum and joining the vitelline veins. The septum is thus shaped like a shield, rounded below to fit the body wall, straight across the top where the umbilical veins run. Dorsal to it or rostral to it, depending on the angle of its slant, the pericardial cavity is continuous with the peritoneal cavity by two wide lateral passages, one on either side of the foregut. These passages are destined to become the pleural cavities and are shown in an older embryo (figs. 2 and 4). At three weeks the foregut has developed two small ventral pouches, one in the region of the passages, one farther down, beneath the septum (fig. 1, arrows). These are to produce respectively the lungs and the liver.

As the embryo grows larger, a third system of paired veins develops (figs. 2 to 4). Of those already mentioned, the vitelline veins represent the mesenteric and portal system of the adult, accompanying the intestine and bringing absorbed food from the yolk sac; the umbilical veins bring food from the placenta. The third system is to produce all the nonintestinal veins of the adult body and carries, not food, but waste products by collecting blood from the small branches of the aorta which supply the body tissues. They therefore run in the dorsal body wall near the paired aortas. To reach the heart and return the blood to the circulation

From Harvard Medical School.

1. His, W.: Anatomie menschlicher Embryonen, Leipzig, F. C. W. Vogel, 1880.

(in which the waste products at this early period may pass to the placenta for elimination), they force a passage at first laterally and then ventrally through the lateral body wall and join the umbilical veins as the latter turn inward to traverse the dorsal edge of the septum transversum. This third paired system of veins is known as the cardinal system and comprises three parts: the anterior cardinal vein collecting blood from the head, the posterior cardinal vein draining the trunk and the common cardinal vein or duct of Cuvier connecting these two with the umbilical system. The horizontal portions of the umbilical veins, which thus become a meeting place for all three venous systems, is called the sinus venosus, emptying into the venous end of the heart.

In the embryo of 4 mm., about 5 weeks, another change has occurred. The liver has grown from the small ventral pouch of the foregut by the sprouting of anastomosing cords of cells directly into the mesoderm of the septum transversum. The cords are accompanied by capillaries from the vitelline veins which become the hepatic sinusoids. The early and rapid growth of the liver is due to its position astride the food-bearing veins, nearest to the source of supply. In contrast the lungs remain merely a pouch. The liver mass spreads ventrally and laterally to the body wall and also dorsally beyond the limits of the septum transversum, on both sides of the foregut, jutting into the pericardioperitoneal or pleural passages but not completely closing them. Since the liver develops in the septum transversum, it is covered by the surface layers of the latter; the cranial layer remains the thicker and is considered as the main septum, from which the liver is suspended, and the caudal layer becomes the hepatic capsule. The dorsal projections of the liver (the dorsal lobes) are also covered cranially by thick layers, which may be called the dorsal extensions of the septum. These are more horizontally set than the septum proper, their two planes making an obtuse angle with the apex at the sinus venosus. They are destined to form an important part of the diaphragm and already mark the lower limits of the pleural cavities.

The upper limits of the pleural cavities are formed through the agency of the ducts of Cuvier, as is shown in an embryo of 12 mm., 7 weeks (fig. 5). By this time the umbilical veins in the lateral body wall have shortened their curved course by moving ventrally, and in the ventral body wall they have thereby come close to the liver with its sinusoids. The two sets of venous vessels unite and form a new, shorter channel, which is enlarged and provides a direct path from the umbilical cord through the liver to the heart. The now unused portions of the umbilical vein in the body wall degenerate, and this releases the ducts of Cuvier from their former anchorage in the lateral walls and allows them to shorten their former curved course around the sides of the passages by drawing across these cavities to the walls of the foregut, with which they soon fuse to form part of the mediastinum. This explains the adult position of the right duct of Cuvier, known now as the vena cava superior; the left duct degenerates after transferring its burden to the new innominate vein. In this mesial migration each duct of Cuvier draws with it across the passage a curtain or fold of the inner layers of the lateral body wall. It happens that the line of the lateral base of the fold corresponds with the course of the ventral branch of the fifth cervical nerve, running in the body wall, and the nerve is thus carried in the edge of the fold to its permanent position in the mediastinum, lateral to the vena cava superior on the right side and at the mediastinal border on the left, whence the nerves may pass to the central part of the septum transversum. The nerve could not grow directly into the slanting septum transversum, as usually stated, for the septum proper never extends to the dorsal wall to receive it. At 12 mm. (fig. 5) the curtain has already fused

with the wall of the gut tract (and is therefore shown as a cut surface) except for a minute tubular gap ventrally, which soon closes and thus completes the separation of the pleural and pericardial cavities.

In this same embryo the septum transversum has become more horizontal than in the younger forms because the gut tract has lengthened caudally more rapidly than the ventral wall of the chest. The increase of liver substance has produced a recognizable supraumbilical abdominal wall. The dorsal extensions of the septum transversum, to which the dorsal hepatic lobes are appended, have been tilted correspondingly caudally, as though the liver and septum were suspended from a transverse line drawn in front of the foregut and swung upward in front, downward behind. The continuing change in position of the liver can be followed in older embryos (figs. 7 and 9). The upper surfaces of the dorsal lobes slant first cranially (at 4 mm.), then successively horizontally and caudally at a rather narrow angle with the dorsal wall. The caudal extent of the pleural cavities is correspondingly increased.

The upper surface of the septum proper at 7 weeks is no longer a flat horizontal plane, for the great enlargement of the heart has bulged its central portion downward, while its attachment to the lateral wall has remained fixed, so that it is forced to assume roughly the shape of the bowl of a spoon or a scoop. In order to understand the future development of the muscular diaphragm it is important to note that the line of lateral attachment follows the course of the first thoracic nerve at the level of the future first rib. Since the dorsal hepatic extensions are not attached to the lateral body walls nor to the wall of the foregut, their upper surfaces remain flat.

The dorsolateral wall of the pleural passage has developed a shallow depression or niche, well marked by an overhanging rim or edge which forms an arch cranially and is continued caudally as two pillars, known as the dorsal and ventral pillars of Uskow. The dorsal pillar forms a narrow vertical ridge on the dorsal wall, mesial to the posterior cardinal vein (figs. 5 and 6). The ventral pillar is attached to the dorsal edge of the septum transversum and to the lateral edge of its extension. As the extension grows dorsally, the pillar is stretched out to form more membrane, which, since the niche is continuous below with the peritoneal cavity, is called the pleuroperitoneal membrane. The niche is not, as sometimes stated, caused by the bulging of the duct of Cuvier into the pericardioperitoneal passage, for the niche is located at some distance below this vessel. As I shall show in another place, it is a persistent member of a linear series of similar niches arising in relation with the series of branchial pouches, many of which, though present in lower forms, have been lost in mammals. The pillars are brought closer together by later changes, and their edges finally fuse to make with the arched portion a continuous membrane. The process is nearly complete at 20 mm. (fig. 7). The pleuroperitoneal membrane in man plays only a minor rôle in the formation of the diaphragm.

Lateral to the dorsal pillar on the dorsal wall of the celom in connection with the posterior cardinal vein, a broad bulging longitudinal ridge develops, caused by the growth of the tubules of the wolffian body or mesonephros, the embryonic excretory organ. This organ arises rather late in embryonic life and continually shifts its position by the caudal addition of new tubules and the degeneration of the older cranial tubules. It shows great variation of size in different animals. In the human embryos here shown it has not yet developed at 4 mm., forms a bulging mass at 12 mm. and has degenerated at this level at 20 mm. and older

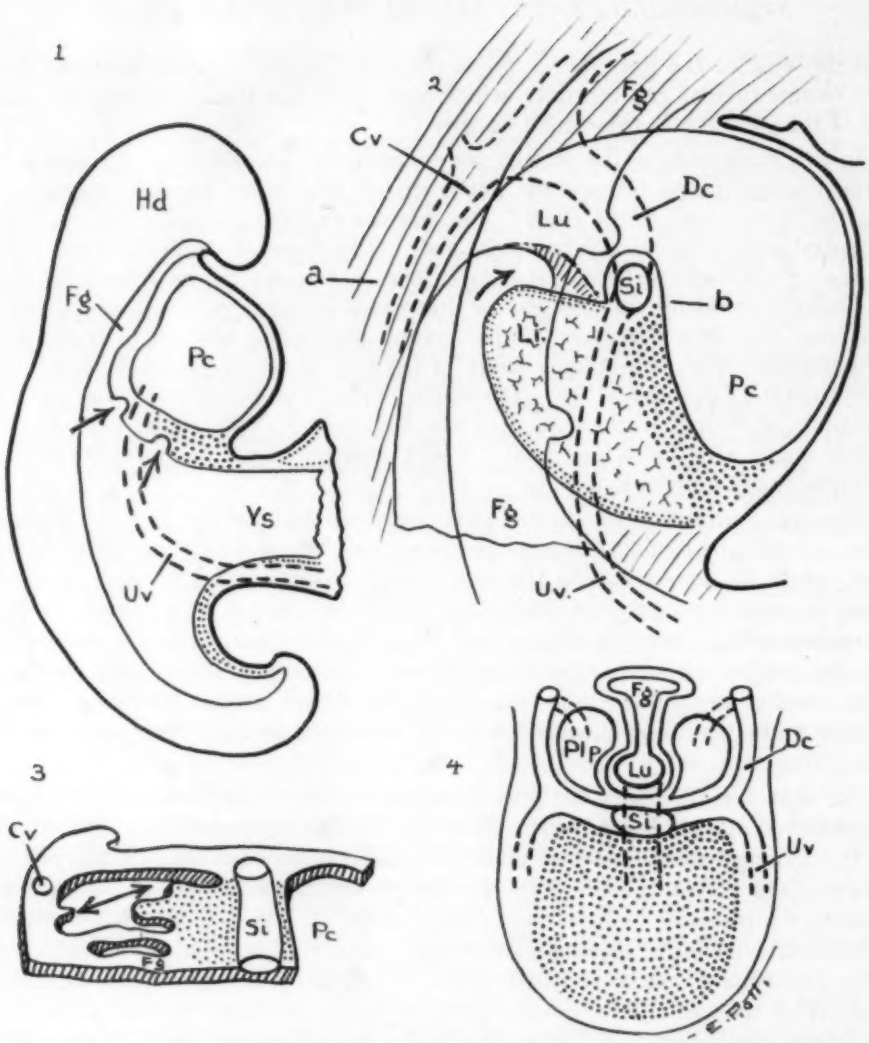

Fig. 1.—Median sagittal section of a human embryo of 24 days. Beneath the head is the bulging pericardial cavity, bordered caudally by the septum transversum (coarse stipple) extending from the ventral body wall just above the umbilical cord to the foregut. The latter runs from the oral plate in the head out into the umbilical cord as the yolk stalk. On its ventral surface are two pockets (marked by arrows) for the lungs and the liver. The umbilical vein runs in the body wall from the umbilical cord to the heart.

Fig. 2.—Paramedian view of the left side of a human embryo of 4 mm. The septum transversum lies more in the vertical plane, and the liver has grown from the diverticulum into its lower border, reaching the ventral wall and extending dorsally as the dorsal lobe, whose flat top is the dorsal septal extension. Above this lies the lung bud in the precardio-peritoneal passage. The arrow points to the parietal niche. The cardial veins run in the body wall, the duct of Cuvier joining the umbilical vein in the sinus venosus. The diagram is based on reconstructions.

Fig. 3.—Section through part of the left side of the same embryo at the level of the line *a-b*. A portion of the pericardial cavity is to the right, then the edge of the septum transversum lodging the sinus venosus. Dorsal to this the septal extension bulges into the pericardioperitoneal or pleural passage, which is bounded laterally by the body wall, mesially by the mediastinum, in which the pharyngeal (foregut) cavity is shown. In the dorsal wall is seen the posterior cardinal vein. Double arrows point to dorsal and ventral pillars, lateral to which lies the niche.

Fig. 4.—Section of same embryo cut in the plane of the septum transversum to show the two comma-shaped pleural passages into which bulges the lung bud from the foregut. The passages lie dorsocranially to the septum, and around their lateral walls run the ducts of Cuvier.

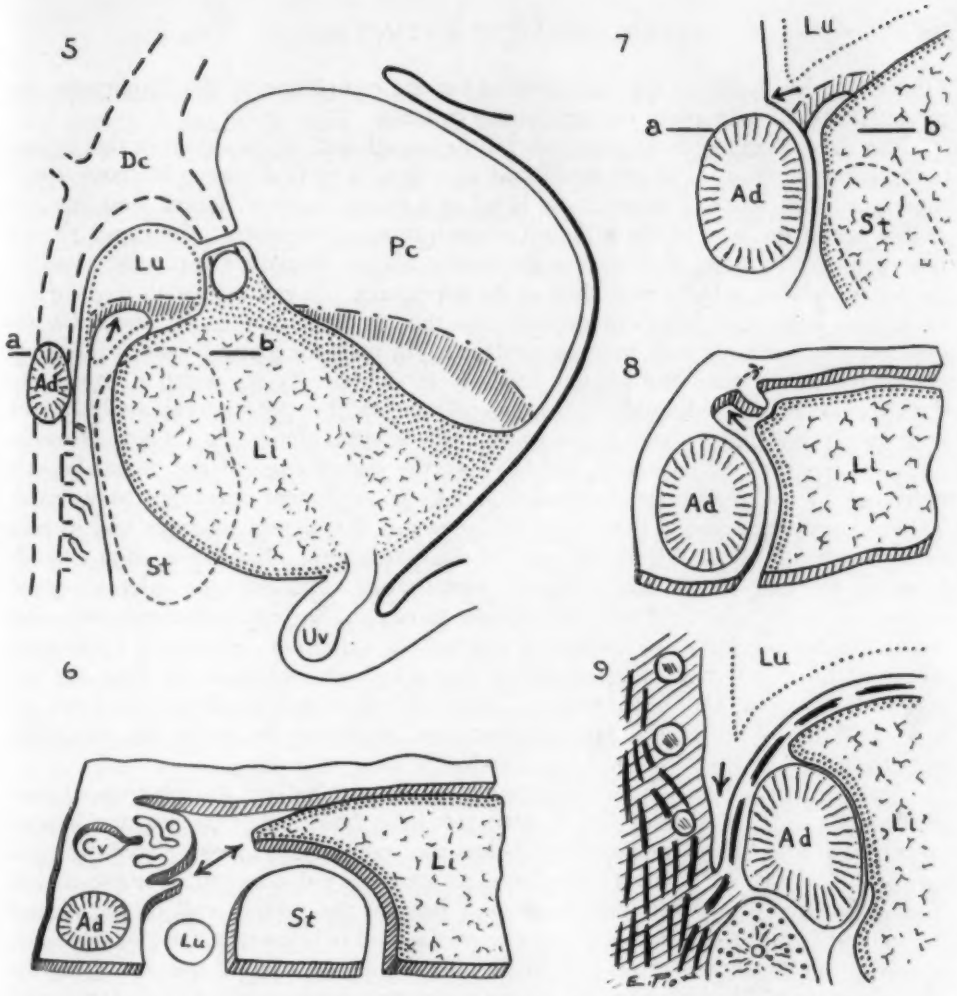

Fig. 5.—A view similar to that in figure 2 of the left side of an embryo of 12 mm. The liver meets the ventral wall on a wider area and receives from it the umbilical vein. The septum transversum lies in a more horizontal plane; its dorsal extension points somewhat caudally. The duct of Cuvier has drawn across the pleural passage to the mediastinum, leaving only a small tubular gap. The pleural cavity is thus delineated and is shown enclosing an enlarged lung (dotted line). The position of the stomach is indicated by a broken line. An arrow points to the niche, the upper limit of which is continuous with the lateral attachment of the scoop-shaped septum. Mesial to the cardinal vein lies the adrenal gland.

Fig. 6.—Transverse section along line *a-b* to show the relative positions of the liver, the stomach, the lung and the adrenal gland. Double arrows point to the pillars of the niche, in the dorsal wall of which are the posterior cardnial vein and tubules of the wolffian body.

Fig. 7.—Similar view of the left side of an embryo of 20 mm., showing the region of the adrenal gland and the pleuroperitoneal membrane. The stomach has descended; the adrenal gland bulges to meet the dorsal hepatic lobe. An arrow points in the direction of the enlargement of the pleural cavity.

Fig. 8.—Transverse section along line *a-b*. The second direction of the enlargement of the pleural cavity is directed along the arrows, outside the dorsal pillar of the niche.

Fig. 9.—Sagittal section of an embryo of 30 mm. to show the splitting off of muscle fibers from the lower ribs and their transfer to the diaphragm.

ABBREVIATIONS USED IN FIGURES 1 TO 9

Cv, cardinal vein
Dc, duct of Cuvier
Fg, foregut
Hd, head
Li, liver
Lu, lung
Pc, pericardial cavity
Plp, pleural passage
Ys, yolk stalk
Si, sinus venosus
St, stomach
Ad, adrenal gland
Uv, umbilical vein

(Felix [2]). Since this is the critical level for the completion of the diaphragm, the mesonephros in man plays no part in the process.

The most median paired structure on the dorsal wall of the celom in this region is the adrenal gland. Not yet developed at 4 mm., it is first shown in these drawings at 12 mm. (figs. 5 and 6). It is an oval organ, retroperitoneal, and situated at this age at the level of the fifth and seventh thoracic vertebrae. Its rapid growth soon causes it to bulge sharply into the cavity, almost blocking the pleural passage; its flat top makes a horizontal shelf at its upper end. In common with most of the embryonic organs, it migrates caudally, so that at 20 mm. (fig. 7) the shelf lies opposite the ninth or tenth thoracic vertebra. In man the gland becomes relatively enormous, larger than the kidney, in early fetal life. By its width it forces the dorsal pillar of the pleuroperitoneal membrane to the dorsolateral angle of the body cavity, causing the membrane to lie in the sagittal plane. At 20 mm., 9 weeks (fig. 7), the shelf at its upper end has met the dorsal edge of the dorsal hepatic extension of the septum transversum, and their peritoneal coverings have fused. This union forms a broad horizontal bridge across the pleural passages and in man is the important factor in the "closure" of the diaphragm. The fusion soon extends laterally to join the pleuroperitoneal membrane. Mesially the adrenal gland widens to reach and fuse with the mediastinum. The only communication still remaining between the two cavities is a small slit on either side of the esophageal mediastinum, for it will be remembered that the dorsal lobes of the liver are not attached to the wall of the foregut. Since the development differs here on the right and left sides of the body, it becomes necessary to study the two sides separately. It is also necessary to introduce a new structure.

Another pair of niches, comparable to those covered by the pleuroperitoneal membranes, is formed in relation with the lung buds. The niches are located on the two sides of the mediastinum, below the roots of the lungs. Over the right recess the dorsal and ventral rims unite progressively downward, forming a continuous membrane, which thus becomes a part of the mesial wall of the pleural cavity and forms the outer layer of the mediastinum below the root of the lung. Caudally the membrane meets and fuses with the mesial edge of the dorsal hepatic lobe along its whole extent. The mesial membrane encloses a long tubular recess, in shape like a flattened finger of a glove, crescentric in cross section to fit the esophagus, and open below into the peritoneal cavity. It was therefore named by Broman [3] the pneumaticoenteric recess. This is normally soon pinched off by pressure of the diaphragmatic muscles, the lower portion becoming the superior recess of the lesser peritoneal cavity; the upper part, if it persists, forms the inconstant bursa infracardiaca (Broman), a structure not described in most textbooks of anatomy but occasionally found in the lower mediastinum.

On the left side a similar recess develops and is present in human embryos during the third and fourth weeks; it then flattens out and disappears. The intestinal tract bends sharply to the left so that the rounded dome of the stomach comes between the mediastinum and the dorsal lobe of the liver. The upper wall of the lobe, which is the dorsal extension of the septum transversum, thus becomes sickle sharped, compressed between the curved body wall and the side of the stomach, but it remains attached dorsally to the shelf on the upper surface of the adrenal gland. The former slit between the septal extension and the mediastinum

2. Felix, W.: The Development of the Urogenital Organs, in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1910, vol. 2, pp. 752-973.

3. Broman, I.: Normale und abnormale Entwicklung des Menschen, Wiesbaden, J. F. Bergmann, 1911.

has thus been enlarged to accommodate the stomach. In this condition the stomach lies directly beneath the lung, with nothing between them (figs. 5 and 6). The closure of this wide gap is accomplished by the caudal migration of the stomach and the growth above it of a thin strip of the left dorsal lobe of the liver and the septal extension to meet the mediastinum, with which the extension then fuses.

The separation of the pleural and the peritoneal cavity is thus accomplished by several independent fusions or adhesions. Similar permanent adhesions over broad surfaces are, of course, made by the mesenteries after the rotation of the intestinal tract. Why they occur and why they are limited to certain definite localities are questions still unsolved. The problem is discussed at length by Broman,[4] but he comes to no conclusions.

The structures which close the pleural passages constitute, however, only a small part of the diaphragm. At about the time of the closure the pleural cavities expand rapidly. It would be satisfactory to think of this as the result of pressure from the growing lungs, after the outlets to the larger peritoneal cavity had been blocked, but this is not the case, for the pleural cavity may be of normal size in the presence of an incomplete diaphragm or of agenesis of the lung (Killingworth and Hibbs[5]). The process seems to be initiated by the general expansion of the body wall, which in pulling on the firm attachments of the septal structures leaves a wedge-shaped area filled with loose connective tissue, into which the pleural cavities then grow.

Dorsally the pleural cavity expands downward behind the adrenal gland (in the direction of the arrows in figures 7 and 9) and mesially toward the bodies of the vertebrae, burrowing into the body wall in such a way as to peel off the innermost layer of muscles and their fascia, which are then just becoming distinct. These are to form part of the muscular diaphragm as distinguished from the membranous diaphragm; the adrenal gland then rests on these muscles, that is, on the diaphragm. The excavation extends for a varying distance behind the gland. Lateral to the adrenal gland there is a small but important gap in which no muscles are stripped off, and then the same sort of burrowing process pushes downward, laterally and forward (fig. 7), peeling off the inner muscles of the thoracic wall for a variable distance anteromesially and to the bottom of the rib cage. This muscle sheet is also added to the diaphragm. The small gap normally is closed by the later fusion of the adjacent edges of the two groups of muscles, which leaves a triangular area peripherally, filled by fibrous tissue, known as the trigonum lumbocostale.

The result of this dissection of the thoracic walls can be traced in the final disposition of the thoracic muscles, as was pointed out by Keith.[6] It will be remembered that laterally the septum transversum remained attached to the body wall at the level of the upper ribs. Laterally, then, the stripping process concerns the whole costal extent, and therefore only two muscle layers (of the typical three layers seen in the abdominal wall) remain connected with the ribs laterally, namely, the external and internal intercostal muscles, the homologues of the external and internal oblique muscles of the abdominal wall. The homologue of the transversus abdominis is absent from the ribs, having been transferred to the diaphragm. In two regions the internal layer persists; between the anterior edges of the pleural cavities the rectus abdominis is represented by the transversus thoracis, and in the gap between the two foci for the commencement of the burrowing the subcostal group persists to represent the quadratus lumborum. The crura belong

4. Broman, I.: Ergebn. d. Anat. u. Entwcklngsgesch. **15**:331, 1906.
5. Killingworth, W. P., and Hibbs, W. G.: Am. J. Dis. Child. **58**:571 1939.
6. Keith, A.: Human Embryology and Morphology, ed. 4, New York, Longmans, Green & Co., 1921.

apparently to the antevertebral group, characterized by attachment to the bodies of the vertebrae and with minor attachments to the transverse processes, represented in man by the psoas muscle below and the longus colli above. The stripping process is shown in the drawing (fig. 9) from a paramedian section of an embryo of 30 mm., 11 weeks, in which the inner layer of the muscles below can be followed into the diaphragm, leaving the transverse processes above the diaphragm bare. This transfer of the crura explains the gap in the lower thoracic region between the vertebral attachments of the longus colli and the psoas.

This description of the derivation of the muscles of the diaphragm differs from that given in many textbooks, in which muscles of the fourth or fifth cervical segments or, in another version, premuscle masses from the lower ends of the infrahyoid groups (Lewis [7]) are said to migrate along with the phrenic nerves while the diaphragm is still opposite the upper cervical vertebrae. If such premuscle masses exist, they must soon degenerate or migrate further, for the adult phrenic nerves enter nonmuscular portions of the diaphragm. The innervation of originally thoracic muscles by a nerve of the fifth cervical segment, apparently alien to them, is not really remarkable when one considers the spread of nerve fibers in the cervical and lumbar plexuses and the overlapping of nerve terminations throughout the body wall. The successful results of surgical transplantation of nerves to new connections are also well known. Any muscle supplied by the ventral rami of other spinal nerves may be taken over by the phrenic, itself a ventral ramus. Even the crural muscles may be taken over, for their homologues, the psoas and the longus colli, being preaxial muscles, are governed by branches of ventral rami. As if to prove their origin definitely, the lateral diaphragmatic muscles still retain "correctly" a minor supply from the thoracic nerves.

It should be emphasized that this description of the closing of the diaphragm applies primarily to man. The great size of the adrenal gland is apparently characteristic of human, or perhaps primate, embryos. In the pig embryo this gland is small and the wolffian body enormous, and the dorsal septal extension meets the larger organ of the dorsal wall. In the rat, on the other hand, both organs are very small, so that the dorsal pillar of the lateral niche retains its position near the mediastinum, spreads diagonally across the passage and in this position meets the edge of the dorsal extension of the septum transversum. The conditions found in other animals have apparently often been transferred to descriptions of the human diaphragm. The field of comparative embryology is almost unexplored, but enough is already known to show the danger of such transfers.

II

A summary or condensation of the embryology of the origins and development of the diaphragm in man as given in the previous pages may serve also as a necessary introduction to the study of the various kinds of diaphragmatic hernia. The diaphragm in man consists primarily of the septum transversum, a sheet of connective tissue which in very young embryos forms the caudal limit of the pericardial cavity and stretches from the ventral and lateral body walls to the ventral wall of the foregut (later esophagus). Behind the dorsal edge of the septum on either side of the foregut the pericardial cavity connects freely with the peritoneal cavity. The two passages thus formed are to lodge the lungs and may be called the pleural passages. Each becomes cut off above from the pericardial cavity by the

7. Lewis, W. H.: The Development of the Muscular System, in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1910, vol. 1, pp. 454-522.

simple growth from the lateral wall of a thin membrane, which ultimately reaches and fuses with the lateral wall of the foregut. The lower closure of the pleural passages is more complicated.

Into the septum transversum grows the liver, an outgrowth from the foregut. It rapidly spreads, ventrally to the body wall and dorsally, as the two dorsal lobes, into the two pleural passages. Everywhere it splits the septum into a thick upper and a thin lower sheet. The portion covering each dorsal lobe is tongue shaped, continuous at its base with the septum, otherwise lying free in the pleural passage which it nearly fills. This part I have called the dorsal septal extension. The fusion of the three edges of the extensions, dorsal, lateral and mesial, with the body walls and mediastinum to effect complete closure is, however, not simple. The dorsal edges fuse with the tops of the adrenal glands, which, being large and precocious in man, bulge forward from the dorsal wall to meet them. The lateral edges meet the pleuroperitoneal membranes, structures which form the covering walls of shallow niches in the lateral walls. The two mesial edges have differing histories. On the right side a tubular niche develops in the wall of the mediastinum, lying in the pulmonary ligament, and the septal extension meets and fuses partly with the mediastinum and partly with the membrane covering this niche. On the left side the stomach swings far to the left, crowding aside the dorsal lobe of the liver and its septal extension. Only after the stomach has descended lower in the abdomen do the hepatic lobe and the septal extension grow over it. The extension then fuses with the mediastinum directly.

The septal structures form only the membranous portion of the diaphragm. The muscular parts are formed by the burrowing of the expanding pleural cavities into the body wall in such a way as to strip off the inner layer of muscle and fascia, which then form the peripheral part of the diaphragm. The burrowing starts from two points, with a slight gap between, and proceeds in two directions, first from behind the adrenal gland, pushing caudally and mesially, and second from the dorsolateral angle of each cavity extending around the ribs to the anterior thoracic wall. The muscles peeled off by these two separate processes later fuse edge to edge, leaving at the gap a triangular area peripherally, later closed by fibrous tissue and known as the trigonum lumbocostale.

The completed diaphragm is thus a complicated structure, the result of a long series of developmental processes of diverse nature. In such circumstances it is almost axiomatic embryologically that the later steps in the process will be most subject to anomalous changes, as though nature lacked the energy to complete the task. Anomalies thus caused are said to be due to arrest of development. One might expect, therefore, that the most common form of diaphragmatic hernia would be through the trigonum lumbocostale, either as a true hernia forcing the fibrous tissue to bulge into the pleural cavity or as a prolapse of abdominal organs through the enclosed triangular gap, called the foramen of Bochdalek. The organ most commonly involved will be the colon, ascending or descending, though other organs may of course be drawn in also. The diaphragm may in other respects be normal, but not infrequently the foramen of Bochdalek is found expanded toward the membranous diaphragm either by the splitting of the edge to edge fusion of the two sets of muscles or by their original failure to unite. This may lead to a long wedge-shaped hole running radially from the centrum to the body wall, through which many of the abdominal organs may pass—stomach, spleen, intestines on the left, intestines and liver on the right. This anomaly is also incorrectly

known as hernia through the hiatus pleuroperitonealis and was so described by Harrington [8]; it is the most common type of all.

The next most common diaphragmatic hernia is in the region of the tendinous centrum on the left side near the esophagus; the organ most frequently involved is the stomach. This has been called hernia through the dome or through the esophageal hiatus. It is due to the failure of the last step in the closure of the pleural passage, the growth above the descending stomach of the thin strip of liver and septal extension to join the mediastinum. Except for the large opening the diaphragm in this type is normal.

Other sites of hernia may be provided by failure of other edges of the protruding dorsal septal extension to fuse with the body wall. Failure to join the adrenal shelf would lead to malformations of the lumbar sector of the diaphragmatic muscles, which, not being pulled away by diaphragmatic attachment, would remain, with the gland, on the dorsal wall. Complete absence of one or both dorsal lobes produces *hernie en croissant,* in which only the septum transversum proper and its appropriate musculature from the rib cage are present. Through such large openings many of the underlying organs could pass.

The two sets of niches with their covering membranes may be the source of another type of diaphragmatic hernia. If the membrane of one of the lateral pair remains incomplete or the niche lies at a higher level than usual and thus exposes more of the weak membrane in the pleural wall, the conditions are favorable for prolapse or true herniation. The position will be at the border of tendinous and muscular portions dorsolaterally. It will be lateral to the dorsal lobe and hence lateral also to the coronary or triangular ligaments, or to a line extending them in the same direction. The primary organ involved may well be the angle of the colon, and the anomaly may occur on either or both sides. In contrast, hernias due to anomalies of the niches and covering membranes below the roots of the lung occur only on the right, for the left niche disappears early. Invasion of the infracardiac bursa by abdominal organs is rare and depends on some peculiarity of the crural muscles. A bulging membrane will form a true hernia through the pulmonary ligament; an incomplete or ruptured membrane, a prolapse. The hernia may be limited to the mediastinum. Since the bursa in these cases is a continuation of the superior recess of the lesser peritoneal cavity, the organs primarily involved will be the duodenum, the bile ducts and possibly the head of the pancreas.

The foregoing are all diaphragmatic hernias due to imperfect completion of the various steps of a normal developmental sequence and therefore properly to be called congenital. Another group is made up of those due to traumas, sustained during birth or later in life, which are either external or muscular. The causes range from rupture of the diaphragmatic muscles to disarrangement of the muscular attachments to the vertebrae or the ribs. The central tendon does not seem to be affected. In such cases the hernia may penetrate a pleural cavity or be limited to the underlying soft tissues. In these cases the special type of attachment of the diaphragmatic muscles is chiefly responsible; the attachments of the crura will give the best illustration of the principle involved. The main origin of each crus is from the ventral surface of the body of a vertebra, but there are minor origins from the transverse processes and from the fibrous loop between the two. This is comparable with the attachments of the longus colli group and is a necessary disposition in a series of overlapping muscles. The psoas muscle passes under the loop, to which it is joined by its delicate fascial investment. The other muscles of

8. Harrington, S. W.: Quart. Bull., Northwestern Univ. M. School **15**:157, 1941.

the diaphragm are similarly attached to loops between the rib tips and interdigitate with the inner abdominal group. The most ventral loop covers the costoxiphoid or Larrey's space. Such a disposition serves well as a barrier between cavities only as long as the pull of the diaphragmatic muscles at the periphery is relatively parallel to the abdominal wall, as is normally the case. If the angle becomes too broad, as it might in extreme muscular action, and quite easily at the costoxiphoid space, the fibrous loop will be lifted from the muscular belly beneath, and the delicate tissue may be torn or weakened sufficiently to permit the penetration of an abdominal organ. The occasional hernia described as penetrating around a nerve or a small blood vessel that perforates the diaphragm depends chiefly on the weakening of the accompanying fibrous tissue. In both these cases some general disease may be a contributing factor by causing degeneration of the binding structures, and it is interesting to notice the recent reports of the coexistence of hernia and anemia (Murphy [9]).

SUMMARY

The summary of the development of the diaphragm in the first paragraphs of part II shows that the last step in the membranous portion is the fusion of the two sets, costal and lumbar, of diaphragmatic muscles peeled off from the body wall. A hernia in this region may be confined to the trigonum lumbocostale and may be either a true hernia or a prolapse of organs; or a perforation may extend between the muscles to the central tendon, allowing a prolapse of many organs. Slightly less common will be a prolapse of the stomach through the central tendon to the left of the esophagus, representing failure of the last step in the adhesions of the septal extensions. In both of these types the rest of the diaphragm may be normal, though distorted. Failure of the septal extension over the dorsal hepatic lobe to fuse with the walls of the pleural passage may cause absence of the lumbar diaphragmatic muscles. Total absence of the septal extensions leads to *hernie en croissant*.

Less frequently a hernia, true or in the form of a prolapse, may be caused by irregularities in the growth of the pleuroperitoneal membrane, to be recognized by its position lateral to the dorsal lobe of the liver, or by failure of the normal cutting off by the crural muscles of the infracardiac bursa (of Broman), thereby leaving an open communication from the superior recess of the lesser peritoneal cavity into the right pulmonary ligament, beside the lower part of the esophagus. Here the organs primarily involved will be the bile duct, the duodenum and the pancreas, though as elsewhere other organs may follow. These are the true congenital hernias.

Traumatic hernias, whether from external violence or excessive muscular action, range from rupture of the muscles to tears at their peripheral attachments, to which their form of attachment by fibrous loops or arches loosely bound down to the underlying abdominal muscles makes them peculiarly subject if the diaphragmatic dome is sufficiently flattened. Other diaphragmatic hernias are similar to those in other parts of the abdominal wall.

9. Murphy, W. P.: Bull. New England M. Center **4**:7, 1942.

AUTOPSY STUDY OF CEREBRAL MALARIA WITH SPECIAL REFERENCE TO MALARIAL GRANULOMA

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Cerebral malaria is one of those manifestations of severe malarial infection which, if not specifically treated in a prompt manner, leads to death in a short while after the onset of the disease. In a number of cases death has taken place before a proper diagnosis was reached. Even at autopsy the diagnosis is likely to be missed in some cases if a histologic examination of the brain is not undertaken. Although the changes in the brain visible to the naked eye in these cases have been well commented on, their histologic characters appear to have attracted the attention of few workers. This is indicated by the meagerness of literature available from this point of view. Margulis and Dürck studied these lesions in detail, and since the appearance of a paper by the latter author in the year 1925 we have been unable to find any reference to a study of the microscopic lesions in cerebral malaria. They described for the first time lesions of the nature of malarial granuloma in the subcortical region of the brain. Dürck [1] offered the explanation that they represented a defense process of an inflammatory nature. We have studied these lesions, and in this paper an attempt is made to present the postmortem observations in the cases of cerebral malaria admitted to King Edward VII Memorial Hospital, Bombay, from the year 1926 to 1941 with special reference to the pathologic changes of the nature of malarial granuloma in the brain in these cases.

MATERIAL

During the course of fifteen years a record was obtained of 97 cases of malaria on which autopsies were performed. Of these cases 55 showed malarial parasites in the capillaries of the brain and were diagnosed post mortem as instances of cerebral or of acute malaria. These 55 cases comprise the basis of this study.

The distribution of the cases as to the years of occurrence is shown in table 1.

TABLE 1.—*Distribution of Cases of Cerebral Malaria as to the Years of Occurrence*

Year	Cases	Year	Cases
1934	7	1938	10
1935	9	1939	12
1936	6	1940	2
1937	4	1941	4

The maximum number of cases occurred in 1939. In this year there was a heavy epidemic of malaria in Bombay. It was attributed to the increase in the breeding places of Anopheles stevensi, the local carrier of malaria, due to irregular rains. This epidemic prevailed for about four months, from July to November.

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1. Dürck, H.: München. med. Wchnschr. **68**:33, 1921.

CLINICAL FEATURES

Of the 55 patients 49 were males while 6 were females. Their grouping according to age periods is shown in table 2. The youngest was a child of 5 years and the oldest a man of 60. The common age period in this series was that between 21 and 40 years, males being more affected. As a majority of the patients were admitted moribund and died soon after, the clinical notes as to previous history have been deficient in many respects. In 35 cases the duration of illness was not mentioned. In 18 it varied from a sudden onset to twelve days, while in 2 the fever lasted for two and one-half months, with marked weakness and cough.

The main clinical manifestations in these cases were distributed as shown in table 3. In 7 cases there was no fever, but the patients were admitted in collapse and died in a short time. This would explain the absence of fever. In all others

TABLE 2.—*Distribution of Cases by Age Periods*

Age Period	Cases	Age Period	Cases
0 to 10 years	1	31 to 40 years	15
11 to 20 years	4	41 to 50 years	10
21 to 30 years	19	51 to 60 years	6

TABLE 3.—*Incidence of Clinical Manifestations in Fifty-Five Cases of Cerebral Malaria*

	Cases
Fever and coma	15
Fever and coma, rigidity of neck, exaggerated jerks, Kernig's sign	10
Collapsed without fever at admission	7
Fever and coma with patient in collapse at admission	6
Fever and delirium	4
Fever and pneumonic signs	3
Fever and drowsy states	2
Fever and paresis of limbs	2
Fever and convulsions	1
Fever and aphasia, exaggerated jerks	1
Fever and dysenteric symptoms	1
Fever and other vague symptoms	3

fever was present. The symptom complex depends on the extent of brain tissue involved and the severity of its involvement. The common symptoms exhibited were fever and coma, which were present in 28 of the 55 cases. The localization of the cerebral condition and of infection of other organs in addition was indicated by such symptom complexes as paresis of limbs, aphasia, dysentery and pneumonia. Fever, coma, rigidity of the neck and exaggerated jerks led to a suspicion of meningitis, to exclude which examination of the cerebrospinal fluid was done in 18 cases. In 9 of them the fluid was normal, while in the remaining 9 it showed a slight increase in the number of cells and in the protein content. In 1 case it was frankly blood tinged. Red blood cells are often found in the centrifugate, and in 1 case the red cells showed the malarial parasites.

ANTEMORTEM DIAGNOSIS

In 27 cases the clinical diagnosis was not entered on the form for the case history, probably because an autopsy was desired. If the clinical diagnosis was put down, there were difficulties in obtaining permission to carry out a postmortem examination. In only 10 of the remaining 28 cases was a definite diagnosis of

cerebral malaria arrived at, while in the remaining 18 cases the conditions listed in table 4 were diagnosed.

The variety of diagnoses indicates the difficulties of arriving at a correct diagnosis. They may be due to (1) frequent absence of malarial parasites in the peripheral blood (referred to again on page 11), (2) varied manifestations of the disease, or (3) insufficient time for investigations necessary to exclude other conditions since most of the patients were admitted in a moribund condition and succumbed within a few hours of admission.

POSTMORTEM OBSERVATIONS

As a majority of the patients suffered from high fever and died shortly after, the viscera in general showed cloudy degeneration. Every spleen was enlarged except one, which was small, weighing 7 Gm. The spleens were firm in consistency and on cutting showed dark brown pulp. Smears were made from the splenic pulp in every case as a routine to exclude malarial infection.

TABLE 4.—*Clinical Diagnoses Recorded in Eighteen Cases of Cerebral Malaria*

	Cases
Tuberculous meningitis	3
Meningitis	1
Pneumonia	3
Gastroenteritis	1
Myocardial failure	2
Anemia	2
Typhoid fever	1
Septicemia	2
Opium poisoning	1
Bacillary dysentery	1
Uremia	1
Total	18

TABLE 5.—*Distribution of Types of Plasmodium in Thirty-Three Cases of Cerebral Malaria*

	Cases
P. falciparum	25
P. vivax	7
P. malariae	0
Mixed	1

In all the 55 cases malarial parasites were found at the autopsy, but the variety of Plasmodium was mentioned in 33 cases only. In these cases the distribution of the three types was as shown in table 5.

Liver.—In 7 cases the liver showed marked cloudy degeneration and slaty-gray discoloration, and in 3, well developed fatty changes. In 22 cases malarial parasites were seen in the capillaries and masses of pigment in Kupffer cells.

Kidneys.—A cloudy change was noted in the renal tissue in 16 cases, while in 14 malarial parasites were seen in the capillaries.

Lungs.—Bronchopneumonia changes were present in 12 cases. Malarial parasites were found in the lungs in 6 cases.

Brain.—From the postmortem notes it is found that the external appearance of the brain was abnormal in 43 cases. The changes visible to the naked eye in these cases are listed in table 6.

In the remaining 12 cases the external appearance was normal. This indicates the necessity of carrying out a microscopic examination of the brain before ruling out cerebral infection, particularly in those cases in which clinical manifestations point toward that possibility. The most common abnormal change is the slaty-gray discoloration of the brain substance due to accumulation of malarial pigment as a result of repeated attacks. This type of discoloration is an evidence of old standing malarial infection on which an acute attack or a relapse is superimposed. Engorgement of blood vessels is suggestive of mild infection, while vascular thrombosis and hemorrhage are an index of greater damage. In 15 cases the vessels of the pia mater were engorged, and the meninges showed evidence of edema and thickening. From the appearance of the naked eye alone it is not possible to predict whether on microscopic examination the brain would show the presence of malarial granulomas in it.

TABLE 6.—*Macroscopic Changes in the Brain in Cases of Cerebral Malaria*

	Cases
Dark and slaty coloration	19
Engorgement of blood vessels and congestion	15
Hemorrhages on the surface	5
Thrombosis of surface blood vessels	2
Edema only	2
Total	43

TABLE 7.—*Distribution of Three Types of Lesions of the Brain in Cases of Cerebral Malaria as to Years of Occurrence*

Year	Granuloma	Necrosis	Hemorrhages
1934	..	2	1
1935	1	2	..
1936	3	..	..
1937	1	1	..
1938	2	..	..
1939	1	..	1
1940	..	..	..
1941	2	..	..

Histologic examinations of the sections of the brain in all these cases showed the capillaries filled with malarial parasites and their pigment. The number of infected red blood cells varied; in some instances they were few, while in others practically every red cell was found to be parasitized. The amount and the character of the pigment present in the red cells or the endothelial cells of the capillaries showed great variation. In some it was present to a marked extent and was coarsely granular, while in others it was scanty and fine.

In addition to the parasites in the capillaries, in 17 cases changes indicative of greater damage to the brain were encountered. In 2 cases were noticed minute punctiform hemorrhages in the subcortical region. They were perivascular, ring shaped, showing in the center an engorged capillary containing infected red cells. The red cells in these hemorrhagic areas were generally nonparasitized but occasionally contained parasites as shown in figure 1 *A*.

In 5 cases subcortical areas of focal necrosis were seen. Each of these had an occluded capillary in the center surrounded by a zone of necrosis which was further surrounded by a zone of extravasated red blood cells.

There was no evidence of glial proliferation in either of the two types of lesions described. In the remaining 10 cases lesions of the nature of malarial granuloma were seen. Table 7 shows the distribution of the three types of lesions

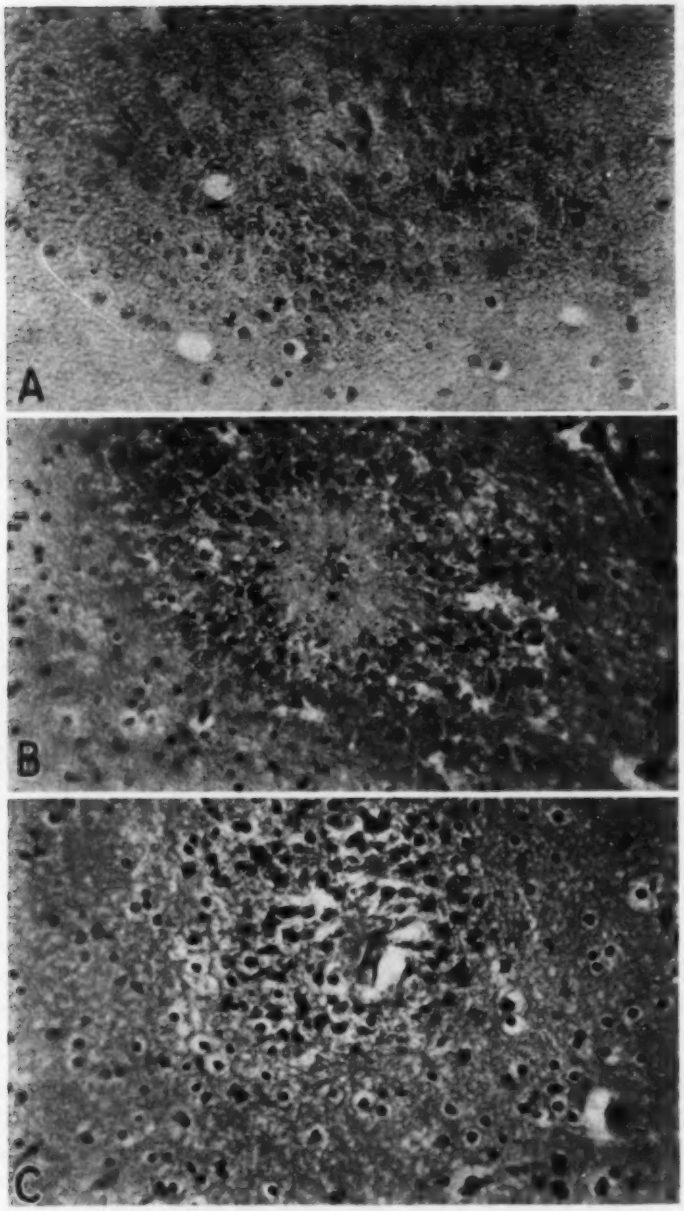

Fig. 1.—*A*, area of hemorrhage. The black dots which are contained in the red cells are malarial parasites.

B, three zones of the typical malarial granuloma. The central black masses are the pigment of the parasites in the occluded capillary. Surrounding it is the necrotic tissue, which looks whitish. Still farther out is an irregular zone of crowded cells—extravasated red cells and proliferating neuroglial cells.

C, well developed malarial granuloma. A distorted capillary is seen in the center and surrounding it is a mass of newly formed neuroglial cells.

according to the years of occurrence. It must be noted that cases in which granuloma was present showed in addition a few areas of hemorrhages or focal necrosis but those in which hemorrhages or focal necroses were numerous did not show any granulomatous lesions. Table 7 shows that in the year 1939, when there was a severe epidemic, only 1 case with granulomatous nodules occurred. The significance of this finding is discussed in the following section.

MALARIAL GRANULOMA

A typical malarial granuloma shows three zones (fig. 1 *B*): First one sees a centrally situated occluded capillary containing pigmented, parasitized red cells. The endothelial cells lining the wall of the capillary have a swollen appearance, and in some cases evidence of proliferation can be detected. Second, immediately surrounding the occluded capillary, a zone of necrotic tissue is seen. This appears as a fairly broad zone of an amorphous pinkish material in which cell structures cannot be identified. Third, farther out, an irregular zone of extravasated red cells is seen, and in the midst of these are a number of proliferating neuroglial cells with an occasional microglial element. In some cases these proliferating cells form a ring between the necrotic zone and the mass of extravasated corpuscles. Granulomatous nodules which are older do not show these three zones. In them a central capillary is surrounded by a mass of nuclei of the neuroglial cells with

TABLE 8.—*External Appearance of Brain in Cases of Cerebral Malaria*

	Cases
Slate-gray discoloration	4
Hemorrhagic exudate on the surface	1
Engorgement of blood vessels	3
Normal appearance	2

little of necrotic material (fig. 1 *C*). This zone of crowded cell nuclei gives these areas a characteristic appearance.

The number of lesions in sections from the same brain varied considerably. In some sections a few diffusely scattered areas were seen in the subcortical region. In others the lesions were so numerous that almost every field under the low power showed one or two. We have sections in our collection in which even under the high power two or three lesions are seen in one field. In some cases all the changes mentioned, occlusion of the capillary, punctiform hemorrhages, focal necrosis and malarial granuloma, were seen in one section; in others more than one section had to be cut in order to detect them. It is significant to note that all these changes were restricted to the subcortical region, the cortical tissue showing only engorged capillaries containing parasitized pigmented red blood cells.

Plasmodium falciparum was the infecting type of parasite in 8 of the cases in which granuloma was noted. In the remaining 2 the type was not mentioned in the postmortem notes. Unfortunately, the clinical notes do not mention whether the patients had suffered from previous malarial attacks. In 4 of them the disease lasted for about ten days. The fact that the spleen was markedly enlarged at autopsy in every one of them indicates that they were subjects of a chronic malarial infection on which the terminal attack, which in a majority of them was of short duration, was superimposed and led to a fatal issue.

It has been mentioned that in the year of the epidemic, when there was an opportunity to study 13 brains, only 1 showed malarial granuloma. The reason for it probably is that granuloma, which indicates a neuroglial response, takes

some time to be produced and therefore is likely to be found in the brain of a person who has suffered from an attack of cerebral malaria but has not succumbed to it. In an epidemic the cases are fresh, the patients die quickly and there is no time for the formation of granulomatous lesions.

The external appearance of the brain in cases showing malarial granuloma was as shown in table 8.

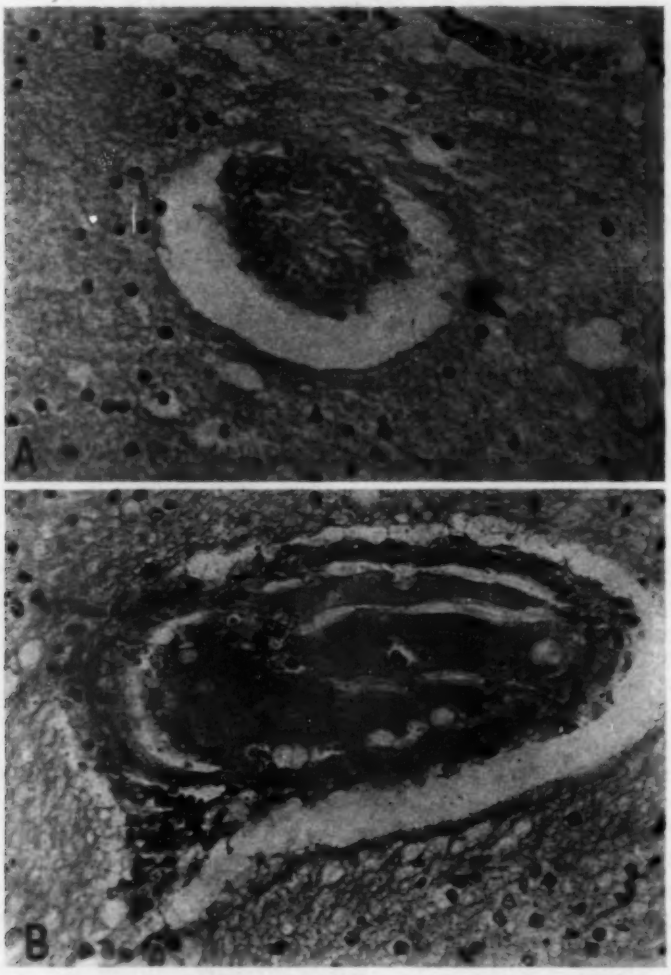

Fig. 2.—*A*, thrombosed blood vessel showing parasitized red cells adhering to the wall. *B*, a large blood vessel in which at one place parasitized red cells are agglutinated and are adherent to the vessel wall.

The lesions described indicate that malarial parasites (especially P. falciparum) are capable of producing severe damage to the nervous system. The parasitized red cells have a tendency to agglutinate and adhere to the vessel wall (fig. 2 *A*). The endothelial cells swell and proliferate. These proliferated endothelial cells together with the agglutinated infected red cells fill and obliterate the smaller blood vessels by a process of thrombosis. Not all the affected blood vessels, however, show evidence of thrombosis. In the larger ones there is produced an obstruction

to the blood flow (fig. 2 *B*). Through the damaged capillary wall diapedesis of red cells takes place giving rise to perivascular, ring-shaped, punctiform hemorrhages. If there is complete occlusion of a number of capillaries in an area, the local circulation is disturbed and areas of focal necrosis of the brain tissue in relation to these blood vessels are produced. If the patient survives, reparative processes come into play. Neuroglial tissue responds by active proliferation, and microglial cells invade the field, infiltrating into the necrotic and hemorrhagic zones. It is this glial response which gives the lesion a granulomatous appearance. Margulis drew attention to these lesions in the year 1914. He found them as nodular areas in persons dying of cerebral malaria. Dürck confirmed his findings and, as stated in our introductory paragraph, brought forth evidence to show that these nodules represent a response to the irritation produced by the malarial parasites and are essentially of the nature of an inflammatory response. In due course they are replaced by a patch of sclerosis representing a mass of newly formed neuroglial tissue.

COMMENT

From the foregoing description it may be seen that in 30 per cent of cases of cerebral malaria the lesions are indicative of severe damage to the brain tissue. Whether the individual lesion is of the nature of a hemorrhage, a focus of necrosis or a granulomatous nodule, the end result is the production of a patch of sclerosis. Multiple areas of sclerosis diffusely scattered would produce disturbance of cerebral function. Wright [2] cited 7 patients suffering from cerebral malaria who had to be admitted to hospitals for patients mentally ill. Three presented a schizophrenic state in which malaria may have played a precipitating role. A confusional state was noted in 3 others. It is mentioned that the pathologic development in these cases is not fully known. It is easy to imagine that lesions of the nature described in this paper would provide the pathologic basis for mental disturbances which generally go under the name of malarial psychosis.

As has been stated, the lesions in our 55 cases were restricted to the subcortical zone. This we presume is explained by the arrangement of the cerebral circulation. On the surface of the brain the blood vessels form intercommunications, but when the vessels enter into the brain substance a sufficient amount of collateral circulation is not present. Occlusion of a stretch of a capillary is likely to produce more damage to the brain in the subcortical region than in the cortex. That appears to be the reason why the lesions are so common in the subcortical region and are hardly ever discovered in the cortex.

It is generally believed that cerebral malaria is produced by P. falciparum. In 7 of our cases the type of malarial parasite found in the splenic smears was Plasmodium vivax and was presumably the type which produced the cerebral involvement. It is the property of P. falciparum to get localized to a particular anatomic region and produce a severe infection of the red cells and damage to the capillaries. This study indicates that on occasions P. vivax may behave in a similar manner. From a clinical point of view it has been observed that in some of these cases in which there was heavy cerebral infection the blood failed to reveal the parasites. This adds to the difficulty of arriving at a correct diagnosis. In our series a record as regards antemortem examination of the blood was found in 15 cases only; parasites were detected in 3 of these. Apart from attaching too much significance to this finding it might be stressed that from a clinical point of

2. Wright, F. J.: East African M. J. **18**:226, 1941; abstracted, Trop. Dis. Bull. **39**:432, 1942.

view the diagnosis of cerebral malaria should not be ruled out on the ground that the parasites have not been found in the blood.

The question whether all the changes mentioned could be explained on the basis of a disturbance of circulation due to blockage of capillaries or whether a toxin was at work, as believed by some observers, has still to be kept sub judice. In this connection the recent work done by Anderson and Morrison [3] on the role of parasite pigment in malarial lesions in monkeys is interesting. Devine and Fulton [4] published observations on the nature of the malarial pigments present in infections of monkeys (Macacus rhesus) with Plasmodium knowlsi. They described chemical and spectroscopic observations which indicate quite clearly that the pigment is indistinguishable from hematin. They produced lesions in the brains of monkeys similar to those observed on infection with P. knowlsi by giving intravenous injections of hematin. Widespread thrombosis was seen in blood vessels, and hemorrhages were produced. Since hematin was not present in a soluble form, they held that the changes produced must be ascribed to the vascular damage produced by the parasites themselves and not to hematin which, because of its insolubility, cannot diffuse out into the brain substance. In the cases herein reported brown pigment was seen outside the capillaries in a greater or lesser quantity in almost every lesion. How far the pigment masses contributed toward the initiation of the reactive processes it is difficult to say.

SUMMARY

An analysis of the postmortem records of 55 cases of cerebral malaria is given, and the lesions of cerebral malaria are described from the standpoint of histology. In 30 per cent of the cases lesions suggestive of greater damage to the brain tissue than would be occasioned by mere occlusion of capillaries were noted, viz., punctiform hemorrhages, areas of focal necrosis and malarial granuloma. The appearance and genesis of malarial granuloma have been studied.

3. Anderson, W. A. D., and Morrison, D. B.: Arch. Path. **33**:677, 1942.

4. Devine, J., and Fulton, J. D.: Ann. Trop. Med. **35**:15, 1941; abstracted, Trop. Dis. Bull. **39**:438, 1942.

SPONTANEOUS AND EXPERIMENTAL ENCEPHALITOZOON INFECTION IN LABORATORY ANIMALS

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Encephalitozoon was first described by Wright and Craighead [1] as the etiologic agent in a spontaneous paralytic disease of young rabbits. Subsequent reports on spontaneous rabbit infections with parasites of a type considered to be the same as that described by Wright and Craighead have been published by Doerr and Zdansky,[2] Levaditi, Nicolau and Schoen,[3] Oliver,[4] Goodpasture,[5] Cowdry and Nicholson,[6] Veratti and Sala,[7] Cameron and Maitland,[8] Da Fano [9] and Smith and Florence.[10] In addition, Levaditi and associates [3b] and Cowdry and Nicholson [11] reported spontaneous Encephalitozoon infection in mice. Levaditi and co-workers [3b] also described experimental transmission of the infection.

While there have been no unequivocal cases of Encephalitozoon infection in man, recent interest in this type of infection has been stimulated by the difficulty encountered in differentiating it from toxoplasmosis; in fact, Olafson and Monlux [12] have suggested that Encephalitozoon and Toxoplasma may be identical. Wolf, Cowen and Paige,[13] Pinkerton and Weinman [14] and others accept them as two types of parasites, but they stress the need for further experimental work on Encephalitozoon with particular regard to differentiating it from Toxoplasma.

In view of the apparent need for further experimental work on Encephalitozoon, the present study was undertaken. A comparative study of Encephalitozoon and Toxoplasma will be reported separately.

SPONTANEOUS INFECTION

In order to gain some idea of the incidence of spontaneous Encephalitozoon infection among certain small laboratory animals used at the National Institute of Health, the experimental material studied in the division of pathology of this institute was utilized. In selected studies in which there was no likelihood of experimentally produced Encephalitozoon infection, a search was made on the routine brain sections of Swiss mice, albino rats of the Wistar strain, guinea pigs and New Zealand white rabbits. The animals were obtained from stock colonies at the National Institute of Health and from commercial breeders; all were apparently healthy

From the Division of Pathology, National Institute of Health, United States Public Health Service.

1. Wright, J. H., and Craighead, E. M.: J. Exper. Med. **36**:135, 1922.
2. Doerr, R., and Zdansky, E.: Schweiz. med. Wchnschr. **53**:1189, 1923; Ztschr. f. Hyg. u. Infektionskr. **101**:239, 1923.
3. Levaditi, C.; Nicolau, S., and Schoen, R.: (*a*) Compt. rend. Soc. de biol. **177**:985, 1923; (*b*) Ann. Inst. Pasteur **38**:651, 1924.
4. Oliver, J.: Arch. Neurol. & Psychiat. **11**:321, 1924.
5. Goodpasture, E. W.: J. Infect. Dis. **34**:428, 1924.
6. Cowdry, E. V., and Nicholson, F. M.: J. Exper. Med. **40**:51, 1924.
7. Veratti, E., and Sala, G.: Boll. Soc. med.-chir., Pavia **37**:45, 1924.
8. Cameron, G. C., and Maitland, H. B.: J. Path. & Bact. **27**:329, 1924.
9. Da Fano, C.: J. Path. & Bact. **27**:333, 1924.
10. Smith, T., and Florence, L.: J. Exper. Med. **41**:25, 1925.
11. Cowdry, E. V., and Nicholson, F. M.: J. A. M. A. **82**:545, 1924; footnote 6.
12. Olafson, P., and Monlux, W. S.: Cornell Vet. **32**:176, 1942.
13. Wolf, A.; Cowen, D., and Paige, B. H.: Am. J. Path. **15**:657, 1939.
14. Pinkerton, H., and Weinman, D.: Arch. Path. **30**:374, 1940.

when selected for experimental use, showing no clinical evidence of any spontaneous infection. Tissues were fixed in a 3.7 per cent solution of formaldehyde or in Orth's fluid, and four or five vertical transverse blocks were cut from each brain; one or two sections were cut from each block and stained by Romanovsky's method as modified by Lillie.[15]

The brains of 502 mice, 283 rats, 291 guinea pigs and 50 rabbits were examined. Encephalitozoon was found in 5 mice, 2 rats and 1 guinea pig, but not in the rabbits. Most of the parasites seen in routine sections were compactly grouped within cells or in round, oval and occasionally lobate cystlike accumulations ranging from 8 to 50 microns in diameter. Free forms were rarely seen, and they were differentiated from granular debris only with great difficulty. When the parasites were intracellular, the parasitized cells were sometimes identified as nerve cells or as macrophages, but more often they were distorted and their type could not be determined. The cystlike accumulations did not exhibit definite walls but appeared to be limited only by adjacent brain tissue. The parasitic accumulations were few in number in 4 of the 8 brains, moderate in number in 3 and fairly numerous in 1. They were irregularly distributed throughout all portions of the brain and were occasionally seen in the meninges.

The parasitized brains all showed an associated inflammatory reaction, with Encephalitozoon being found within or at the margins of lesions and in normal brain tissue at a distance from lesions as well. The parasites within or at the margins of lesions were most often free or within cells, while those in normal tissue were usually grouped in the cystlike accumulations.

In the routine sections stained by the modified Romanovsky method, the parasites were pale blue and difficult to see since they were much the same color as the cytoplasm of some nerve cells. With certain special stains, however, they stained deeply and clearly, and the comparative ease with which they were found was striking. This was especially true of the free parasites within lesions; with the modified Romanovsky stain most lesions appeared to be devoid of the organisms, while with the special stains many lesions were seen to contain them, and frequently they were numerous. The special stains which were found to be most useful were the Giemsa and the stains recommended for Encephalitozoon by Goodpasture[5] (carbol-aniline-fuchsin, decolorized and differentiated with a 37 per cent solution of formaldehyde and counterstained with picric acid (trinitrophenol) and by Wright and Craighead[1] (carbol-fuchsin diluted 1:4, decolorized and mordanted with a 37 per cent solution of formaldehyde and counterstained with methylene blue).

The individual parasites were usually in the form of straight or slightly curved rods with both ends bluntly rounded; one end was usually a little larger than the other, and at times the body was slightly constricted at or near its midpoint. Round and oval forms were observed, but they were much less numerous than the rods. Most of the parasites measured from 0.8 to 1.2 microns in width and from 1.5 to 2.5 microns in length; only rarely did one measure over 2.5 microns. Internal organization could be made out in most of the parasites, particularly with the Giemsa stain. A round, oval or bandlike nucleus was eccentrically placed and was about one-fourth to one-third the size of the parasite. With the Giemsa and modified Romanovsky stains, both the cytoplasm and the nucleus stained blue, but the latter was deeper in color, and the pale cytoplasm often exhibited a clear central area. The parasites stained very deep blue with the stain recommended by Goodpasture and dark red with that suggested by Wright and Craighead; with these stains the parasites were often so deeply colored that it was difficult to differentiate nucleus from cytoplasm.

The extent and the degree of the meningoencephalitis observed in each of the 8 spontaneously infected animals was roughly proportional to the number of parasites found. In the meninges there was slight to moderate focal perivascular infiltration by lymphocytes and a few large mononuclear and plasma cells. No alterations were seen in the choroid plexus, but in the brain substance lesions were irregularly distributed throughout all areas. The lesions of brain most often encountered were perivascular infiltrations similar to those seen in the meninges and small, loose or compact glial cell nodules. The involved vessels were of small to moderate size and at times showed swelling of the endothelium or proliferation of the adventitial cells or both. Nodular granulomatous lesions were seen in 5 of the 8 brains; they were composed of large mononuclear cells with fairly abundant homogeneous eosinophilic cytoplasm and round or elongated leptochromatic and trachychromatic nuclei. Central necrosis was occasionally noted, and at times a marginal accumulation of small glial or lymphoid cells was seen. Foamy macrophages were observed in some granulomatous lesions and in occasional glial cell nodules; at times they were grouped in otherwise normal brain tissue.

In the group of spontaneously infected animals, tissues other than brain were examined from 4 mice and 1 rat. The significant observations were as follows:

The kidneys of 1 mouse showed slight, and those of the other 4 animals moderate or moderate to marked, interstitial lymphocyte infiltration. The infiltration was predominantly

15. Lillie, R. D.: Stain Technol. **16**:1, 1941.

cortical, and in the areas of infiltration the tubular epithelium often showed variable degrees of degeneration or proliferation. Parasites, both intraepithelial and in circumscribed accumulations, were seen in the collecting tubules of the medulla in 1 mouse. There was no inflammatory reaction in the vicinity of the parasites.

All 5 livers revealed a slight increase in the number of cells in the sinusoids, chiefly lymphocytes and large mononuclears, and slight to moderate infiltration of portal areas by similar cells. Four livers had scattered small nodular accumulations of lymphocytes and large mononuclear cells in the parenchyma. A single circumscribed accumulation of parasites was found in a hepatic sinusoid in 1 mouse, and another was similarly located in the rat.

The spleen of every animal appeared to be enlarged in the sections, and there was slight to moderate lymphoid cell proliferation in the follicles and pulp.

Four of the 5 animals showed an inflammatory reaction of slight to moderate degree involving the epicardium. There was irregular infiltration by lymphocytes and fewer large mononuclear and plasma cells, associated with swelling of serosal mesothelial cells, slight fibroblast proliferation and a little collagenization.

Focal involvement of the pleura by a similar inflammatory process was seen in all animals, and the same type of focal reaction, although less marked, was observed in the peritoneum in 4.

The spinal cord, sectioned only in the study of the rat and 1 mouse, showed lesions similar to those seen in the brain, and parasites were found without difficulty. The olfactory bulbs, seen only in the rat, were similarly affected, and the eyes from the same animal showed scattered small perivascular foci of lymphocyte infiltration of the retinas. In the latter, a single accumulation of parasites was found after careful search through serial sections.

No lesions were found in the thymus, which was examined in all animals, or in the vertebral bone marrow, which was seen in 1 mouse and in the rat.

ISOLATION OF ENCEPHALITOZOON

From the observations on spontaneous Encephalitozoon infection it was assumed that stock Swiss mice would serve as a satisfactory source from which to attempt isolation of a strain. The isolation was accomplished by a method combining inoculation and histologic examination. The mice were killed with chloroform, and their brains were removed aseptically. Half of each brain was fixed in Orth's fluid; the other half was suspended in isotonic solution of sodium chloride and used for the inoculation of 4 mice. Two of the mice received 0.03 cc. intracerebrally, and the other 2 were given 0.5 to 1.0 cc. intraperitoneally. From each retained half brain, after fixation, three blocks were cut and from each block 6 to 10 Romanovsky-stained sections were prepared by serially sectioning, mounting and staining every tenth section and discarding intervening sections. Each group of inoculated mice was held until histologic examination had been completed, and then, if the examination had shown no parasites, they were discarded.

Encephalitozoon was found in the brain of the fifty-ninth mouse examined. The 4 mice inoculated from the corresponding half brain were killed at intervals varying from forty-two to one hundred and seven days after inoculation. Encephalitozoon was found in the brains of 2 of these mice. All showed histologic lesions consistent with spontaneous Encephalitozoon infection, and successful transfers were made from each.

An attempt was also made to isolate the parasites from rabbits, using the method described in a foregoing paragraph. Twenty-one white New Zealand rabbits were used; Encephalitozoon was not found, but a strain of Toxoplasma was isolated from 1.

TRANSMISSION

For transmission, peritoneal exudate or suspensions of brain, liver or spleen in isotonic solution of sodium chloride were used; the suspensions were 10 to 20 per cent by weight. Routine inoculations were made intraperitoneally or intracerebrally, and passage by intranasal instillation was successful on both of the trials made. The dose used was 0.03 cc. for intracerebral inoculation, 0.05 cc. for intranasal and 0.5 to 1.0 cc. for intraperitoneal inoculation. When passing a strain from intraperitoneally inoculated mice, brain, liver, spleen or peritoneal exudate was used. Transfers from intracerebrally inoculated mice were nearly always made from the brain, although both of two attempts to use liver and spleen were successful. When liver, spleen or peritoneal exudate was used for inoculums, transfers were made during the second or the third week after inoculation, since this appeared to be the optimum time on the basis of clinical and microscopic examination. Transfers from the brain were usually made three to eight weeks after inoculation, although several passages at later intervals, up to one hundred and eighty days, were successful. Longer intervals are on trial, but this phase of the work has not yet been completed.

Inconclusive results were obtained in attempts to transmit the infection by contact of adult mice. Four inoculated and four uninoculated adult mice were placed in each of three glass jars. The inoculated mice in one jar had received infected material intranasally, while those in the other two jars had been inoculated by intracerebral and intraperitoneal routes, respectively. At three, six, nine and twelve week intervals an uninoculated mouse from each jar was killed. One inoculated mouse from each jar was killed at the end of three weeks, but the remaining 3 were left for the full twelve weeks. All of the inoculated mice had significant lesions, and Encephalitozoon was found in 8. One uninoculated mouse from each jar had a mild meningoencephalitis, but no parasites were found; the remaining uninoculated mice had no evidence of infection.

With infant mice, contact infection was demonstrated, but the exact mechanism is obscure, and further experimental work is indicated. An infected mouse gave birth to a litter of 6 twenty-three days after intraperitoneal inoculation. Two of the litter were killed and examined two days after birth and were without infection. The other 4 were killed a month later; 1 was normal, but the other 3 showed lesions consistent with Encephalitozoon infection, and numerous parasites were found in 2 of these. Another infected mouse gave birth to a litter of 7 three months after intraperitoneal inoculation. All of the litter were killed a month later, and all were without infection.

Two additional experiments were conducted to observe the results if a stock mouse fostered infant mice born of an infected mouse, and vice versa. An infected mouse gave birth to a litter of 6 on the thirty-fourth day after intracerebral inoculation. Two days later the 6 infant mice were given to a stock mouse which had delivered at the same time, and the litter of 5 from the stock mouse was given to the infected mouse. Two of the litter taken from the stock mouse and given to the infected mouse died and were discarded during the first week, while all of the remaining young mice survived until the termination of the experiment at the end of a month. All of the young mice born of the inoculated mouse and fostered by the stock mouse were heavily infected, while the 3 fostered by the inoculated mouse showed no infection. Histologic examination of the inoculated mouse revealed lesions and parasites, while the stock mouse showed no evidence of infection. In the second experiment an infected mouse gave birth to a litter of 10 on the sixteenth day after intraperitoneal inoculation. Two of the young mice were killed as soon as they were found, and both were without infection. The remaining 8 were given to a stock mouse which had delivered the same day, and the 6 newborn mice from the stock mouse were given to the inoculated mouse. Two of the 8 given to the stock mouse were missing the next day, probably cannibalized, but the remaining 6 survived. One of the 6 given to the infected mouse died on the twenty-fourth day and was discarded as unsuitable for histologic examination because of postmortem decomposition. All of the remaining young mice were killed when 2 months of age. The 5 mice delivered from the stock mouse and given to the infected one all had lesions, and numerous parasites were found in 3. The 6 mice born of the infected mouse and fostered by the stock mouse were all normal histologically, and no parasites were found. Histologic examination of the inoculated mouse revealed both lesions and parasites. The stock mouse had lesions consistent with Encephalitozoon infection, and although parasites were not found, they were demonstrated in each of 2 mice inoculated with an emulsion prepared from its brain.

PROPERTIES OF THE PARASITE

Routine cultures of infectious material used in transfers were made on blood agar slants and were negative except for an occasional contaminant. Further attempts to cultivate the parasite were made with acid dextrose agar, dextrose veal infusion agar and a medium which is favorable for the growth of Trypanosoma cruzi[16]; no growth was obtained on any of these mediums.

Tissues from inoculated mice retained their infectivity when preserved at 4 C. in 50 per cent buffered glycerin or Tyrode's solution or when rapidly frozen and stored at —70 C. They were kept for as long as fifteen weeks in glycerin, nine weeks in Tyrode's solution and twenty days when frozen; longer intervals of preservation by these methods were not attempted.

Filtration of infectious material through a Berkefeld N candle was attempted twice. On both occasions the mice inoculated with filtered material were uninfected, while those inoculated with unfiltered portions of the same material showed lesions consistent with Encephalitozoon infection, and parasites were found in smears and histologic sections.

CLINICAL AND GROSS POSTMORTEM FINDINGS

The strain of Encephalitozoon was not very virulent for mice when first isolated, and there was no apparent increase in virulence after many subsequent passages. Although inoculated mice

16. Davis, D. J.: Pub. Health Rep. **58**:2, 1943.

were observed for as long as six months, none appeared to be very ill, and there were no deaths which could be definitely attributed to the parasitic infection.

Mice examined during the first two weeks after inoculation revealed no abnormalities, but during the third and forth weeks varying degrees of abdominal enlargement were noted. The enlargement was slight in those inoculated intracerebrally or intranasally and moderate to marked in those inoculated intraperitoneally. In the latter there was usually evidence of fluid in the peritoneal cavity. The abdominal enlargement persisted for several weeks, gradually decreasing in degree.

At autopsy mice killed within two weeks after inoculation were generally without evidence of infection. Mice killed during the third or fourth week showed enlargement of the liver and the spleen, particularly of the latter, which was often four to five times the normal size. The most marked enlargement of the liver and the spleen was seen in the intraperitoneally inoculated mice; over 95 per cent of these had a clear or slightly cloudy viscid exudate in the peritoneal cavity. The exudate was variable in amount; as much as 8 cc. was removed from an occasional mouse, and 3 to 4 cc. was commonly found. The exudate eventually disappeared in mice kept for several weeks, during which time the spleen became smaller and the liver returned to its normal size.

Alcohol-fixed, Giemsa-stained smears of the peritoneal exudate showed much precipitated granular material and variable numbers of large and small lymphoid cells and mesothelial cells. Encephalitozoon was found in 62 of 77 peritoneal smears prepared from intraperitoneally inoculated mice killed during the third or the fourth week after inoculation. Parasites were not found in the few peritoneal smears examined from mice that were inoculated intracerebrally or intranasally or in those from intraperitoneally inoculated mice killed before the tenth day or after the fifth week.

In the smears the parasites were seldom numerous, and often they were found only after considerable search. Intracellular groups were found more often than extracellular free forms, and while they were occasionally found in the cytoplasm of large mesothelial cells, they were occasionally found in the cytoplasm of lymphoid cells. In the cells the parasites were sometimes irregularly disposed, but more often they were compactly arranged in round to oval accumulations, which were at times of sufficient size to distend and distort the host cells.

The individual parasites in the smears were rod shaped or ovoid; the rod-shaped forms had bluntly rounded ends; one end was often a little larger than the other, and occasionally they were slightly curved. Among the extracellular parasites the rod shapes predominated, but many of those in intracellular groups were ovoid. The vast majority of the parasites measured from 1.0 to 1.8 microns in width and from 1.5 to 3.0 microns in length. The cytoplasm stained pale blue by Giemsa's method, and a clear space often occupied much of the body of the parasite, with condensation of cytoplasm at the periphery. The red to purple or dark blue nucleus usually was one-third to one-fourth the size of the parasite, eccentrically placed, and round, oval, crescentic or bandlike. Binucleate forms were rarely encountered.

A few unidentified intracellular structures were seen in an occasional smear. They were round to oval and larger than the usual forms of Encephalitozoon already described, measuring 4 to 5 microns in greatest diameter. Poorly defined and irregular masses of dark bluish red chromatin material were nearly always multiple, numbering from two to six and occasionally more. The cytoplasm stained a much deeper blue than the cytoplasm of the parasites described in the foregoing paragraphs. These structures were most often found in the cytoplasm of cells which also contained the usual parasitic forms.

HISTOLOGIC OBSERVATIONS

Detailed histologic examinations were done on 147 passage mice, 96 of which had been inoculated peritoneally, 41 intracerebrally and 10 intranasally. They were killed at intervals varying from three to one hundred and seventeen days after inoculation; about half were killed before the sixth week, and the average duration for the remainder was about ten weeks. Thirty-four control mice were examined; 16 were normal stock mice, and 18 had been inoculated intraperitoneally with a suspension of liver in isotonic solution of sodium chloride, in four serial passages from a normal stock mouse.

Histologic lesions were found in all but the 8 mice killed before the eighth day after inoculation. Since in the latter there apparently was insufficient time for lesions to develop, they are excluded in the calculation of percentages. Among the remaining 139 mice, the lesions found were essentially similar in type regardless of the duration or the method of introducing the infection. There were, however, differences in the extent and the degree of some changes, and these will be described with the organs and the tissues concerned.

The most constant and striking histologic finding was meningoencephalitis, which was observed in 93 per cent of the inoculated mice. The incidence and the severity of the process were not affected by the route of inoculation, but the duration of infection exerted a modi-

fying influence; the lesions were more constantly present and generally more severe in mice killed after the fifth week than in those killed in the second to fifth weeks. The lesions for the most part were essentially similar in type and location to those described in spontaneous infection, and the description need not be repeated here. One type of lesion of the brain not observed in spontaneous infection was occasionally noted in the inoculated mice. This was a patchy necrosis, chiefly cortical, in which degenerating nerve and glial cells were set in an oxyphilic, vacuolated and somewhat disorganized ground substance. Lesions of this type were seen in mice inoculated intraperitoneally as well as intracerebrally. Granulomatous lesions were uncommon among the inoculated mice killed during the first few weeks, but they were almost always found in those killed at later intervals. Slight focal lymphocyte infiltration of the choroid plexus was occasionally seen in intracerebrally inoculated mice. Three of the controls showed slight focal perivascular lymphocyte infiltration in the meninges but no parenchymal lesions.

A prominent inflammatory reaction of the peritoneum and retroperitoneal tissues was seen in all intraperitoneally inoculated mice. In those killed within five weeks of inoculation there were swelling and focal heaping of mesothelial cells; monocytic exudate covered portions of the surface, and nodular or patchy infiltration of the retroperitoneal adipose tissue by lymphocytes and large mononuclear cells was noted. Slight to moderate focal infiltration by similar cells was seen in the capsular tissues of the liver, the spleen, the kidneys and the adrenal glands, and in the outer muscularis of the uterus, the stomach and the intestines. In intraperitoneally inoculated animals killed at later intervals the monocytic surface exudate was scanty or absent; swelling of mesothelial cells was slight and focal, plasma cells were often seen in foci of infiltration, and slight collagenization was noted in some lesions. The peritoneal reaction in intracerebrally and intranasally inoculated mice was inconstant and much less prominent, resembling that seen in spontaneous infection. The control mice did not show peritonitis or inflammation of the retroperitoneal tissues.

An inflammatory reaction noted in the pleura and the epicardium of inoculated mice was similar to that described in spontaneous infection. The pleura was affected in 70 per cent of the mice and the epicardium in 60 per cent. The pleural reaction was usually a little more prominent than that seen in the epicardium, and monocytic exudate, rarely noted on the epicardium, was seen focally on the pleura in about 25 per cent of the involved mice. Control mice were not similarly affected.

The alterations in the liver, the spleen and the kidneys were identical with those described in spontaneous infection. The splenic hyperplasia and the hepatic changes were almost constantly present, although variable in degree, with the most marked involvement seen in the intraperitoneally inoculated mice. The renal lesions were seen in 80 per cent of the inoculated mice, and the degree of involvement was not influenced by the route of inoculation. Among the control mice, only 4 showed focal lymphocyte infiltration of the kidneys; the infiltration was chiefly perivascular and parapelvic; however, hepatic alterations similar to those seen in the infected mice were present in a little over half of the control mice, while splenic hyperplasia was seen in a little less than half. The changes in the liver and the spleen in the controls were generally less marked than those observed in infected mice and were most often seen in those which had been inoculated with a suspension of liver.

The adrenal glands of 81 of the infected mice were seen in section. In 32 per cent of these glands slight to moderate focal lymphocyte infiltration was noted. The infiltration was usually cortical, and degenerative changes were seen at times in cortical cells within or adjacent to the lesions. The adrenal glands of 28 of the controls were sectioned, and all were negative for infection.

The lungs of all intranasally inoculated mice showed a patchy pneumonic process of moderate extent, characterized by perivascular, peribronchial and septal infiltration by lymphoid and large mononuclear cells, together with a scanty alveolar exudate composed chiefly of similar cells. A similar but less marked inflammatory process was seen in 66 per cent of intracerebrally and intraperitoneally inoculated mice, but unfortunately a low grade spontaneous infection of the lungs was present in stock mice, and 60 per cent of the controls showed a somewhat similar involvement.

No significant lesions were found in the myocardium, the thymus or the mediastinal or the retroperitoneal lymph nodes of the inoculated mice. Organs which were not routinely sectioned but which were seen in a considerable number of infected mice and were consistently negative for infection included the pituitary gland, the thyroid gland, the testicles and the ovaries. Aside from focal infiltration of the outer muscularis, already noted, the uterus, the stomach and the intestines were without infection.

In the routine histologic examinations, Encephalitozoon was found in 69 of the 139 inoculated mice. They were most frequently found in the peritoneum or the retroperitoneal tissues of intraperitoneally inoculated mice killed three or four weeks after infection; among 54 such mice, parasites were seen in 37. The brains of 137 mice were routinely sectioned and examined,

and parasites were found in 39. Parasites were seldom observed in other organs and tissues; they were found in the pleura in 4 mice, the kidneys in 2 and in the liver, the adrenal gland, the pancreas and the spleen in 1 mouse each.

Since the parasites were very lightly stained and difficult to find in sections stained with the routine modified Romanovsky stain, special methods were used in an attempt to demonstrate them in tissues which had been recorded as negative for parasites on the regular examination. For this purpose, the brains of 32 intraperitoneally inoculated mice killed six weeks or more after inoculation were used; all of these brains had been negative for parasites on routine examination. Six were picked at random from the 32, and serial sections were prepared from the brain blocks and stained with the routine stain; after a persistent search, Encephalitozoon was found in all. Routine sections were then cut from the brain blocks of the remaining 26, but the stain recommended by Goodpasture was used instead of the Romanovsky stain as modified by Lillie. The results were striking! Encephalitozoon was readily found in 22 of the 26.

EXPERIMENTAL INFECTION OF OTHER LABORATORY ANIMALS

The experimental transmission of Encephalitozoon infection to laboratory animals other than mice was attempted on only a small scale. Eight adult Syrian hamsters were inoculated with suspensions of infectious mouse tissue; 4 received 0.1 cc. intracerebrally, while the others were given 1.0 cc. intraperitoneally. All were infected, with clinical and pathologic changes essentially similar to those described in mice; Encephalitozoon was found in 4.

Six adult white rats of the Wistar strain showed no evidence of infection after intraperitoneal inoculation with infectious material in 2.0 cc. amounts, but 3 infant rats of the same strain receiving the same amount intraperitoneally were infected, as were 2 more which were given 0.04 cc. intracerebrally. The clinical and pathologic alterations in the infant rats were again essentially similar to those noted in mice, and parasites were found in 2.

Experimental infection of 3 adult white New Zealand rabbits was attempted by inoculating them in the following manner: The first received 2.0 cc. intravenously, the second 1.5 cc. intravenously plus 2.0 cc. intraperitoneally and the third 5.0 cc. intraperitoneally. All of the rabbits were clinically normal and were killed three months after inoculation, together with a control which had been obtained from stock at the same time as the others. Meningoencephalitis similar in type to that seen in mice was present in the inoculated rabbits, and Encephalitozoon was seen in the lesions in 1 rabbit; the brain of the control rabbit was negative for infection. Other organs and tissues were examined microscopically, but all the rabbits had a coccidial infection, and the significance of the few visceral lesions found was open to question.

Five adult guinea pigs inoculated intraperitoneally with infectious material in 1 to 4 cc. amounts did not show infection clinically or on pathologic examination.

COMMENT

The parasites observed in the spontaneous infections in this study appear to be similar morphologically and in staining reactions to those described in the majority of previous reports of Encephalitozoon infections. However, the parasites described by Veratti and Salla,[7] by Cameron and Maitland[8] and by Da Fano[9] seem to be quite different and are believed to be Toxoplasma rather than Encephalitozoon. The reasons for this opinion will be set forth in a subsequent paper which will deal with comparisons between Encephalitozoon and Toxoplasma.

Spontaneous Encephalitozoon infection, previously described only in rabbits and mice, has, in this study, been found in rats and a guinea pig in addition to mice. In regard to the clinical mildness of spontaneous infection and the predominant localization of parasites and lesions in the central nervous system and the kidneys, the observations made in the present study are in general agreement with previous reports.

Little work on experimental Encephalitozoon infection has been published. A few authors have reported negative results in attempts to transmit the infection by inoculation, while Wright and Craighead,[1] without giving any details, stated that the intracerebral inoculation of rabbits with sedimented urine or emulsions of spinal cords from sick rabbits reproduced the disease. A more extensive and detailed account of the experimental transmission of the infection was given by Levaditi,

Nicolau and Schoen,[3b] who, working almost exclusively on rabbits with strains isolated from the same species, reported successful transmission by the use of brain emulsions or urine, inoculated by various routes. Although they described lesions and parasites in the majority of passage animals (60 to 75 per cent), and control rabbits inoculated with saline solution or with filtrates of infectious material did not show infection, the results obtained by Levaditi and co-workers have been subjected to criticism. In particular it has been pointed out that the experiments were not adequately controlled in regard to ruling out spontaneous infection.

Since the present experimental work was done almost entirely with mice and with a strain isolated from a mouse, a direct comparison of results with those reported by Levaditi and co-workers cannot be made. However, the results do confirm the fact that the disease can be transmitted by inoculation, and this has been the chief point of contention. Other observations made by Levaditi and co-workers and substantiated here are that Encephalitozoon retains its infectivity when preserved at low temperatures in glycerin and that filtered portions of infectious material will not reproduce the disease. On the other hand, the slow development of lesions reported by Levaditi and co-workers in rabbits was not observed in the present study on mice; in the latter the lesions developed much more rapidly.

In the present study the possible complicating factor of spontaneous infections in the experimental mice can be ruled out. There was a low incidence (1 per cent) of spontaneous infections in 502 similar mice from the same sources examined prior to the beginning of the experimental study. Furthermore, the organisms were not found, and there were no lesions suggestive of Encephalitozoon infection in the controls or in mice which were inoculated with Berkefeld N filtrates of infectious material. These findings are in sharp contrast with those in the experimentally infected group; in the latter, lesions consistent with Encephalitozoon infection were constantly present, and routine histologic sections revealed parasites in 50 per cent of the mice.

It may seem strange that the parasites were not found in more than 50 per cent of the experimentally infected mice by routine histologic examination, but in the sections stained with Romanovsky's stain as modified by Lille, the parasites were poorly stained and hard to find. In this regard there is ample evidence to show that parasites were present in the tissues of inoculated mice even though they were not seen. When inoculation records were checked with histologic observations, it was found that numerous transfers had been made from tissues which had lesions consistent with Encephalitozoon infection but in which parasites had not been found; following each of these transfers, Encephalitozoon was always demonstrated among the first passage mice. Even more convincing were the results obtained by serial sectioning or by special staining of tissues which had been negative for parasites on routine examination. By these procedures, a group of 32 brains which had been negative on routine examination were reexamined, and parasites were found in 28.

SUMMARY

The routine experimental material studied in the division of pathology of the National Institute of Health disclosed spontaneous Encephalitozoon infection in certain small laboratory animals. In selected studies in which there was no likelihood of experimentally produced infection, Encephalitozoon was found in 5 of 502 white Swiss mice, in 2 of 283 albino rats of the Wistar strain and in 1 of 291 guinea pigs; 50 rabbits were negative for Encephalitozoon. All the infected animals

exhibited meningoencephalitis, while histologic lesions in other organs and tissues were less striking and constant.

A strain of Encephalitozoon was isolated from the brain of a white mouse and successfully transmitted in series to other white mice. In the serial transmission, peritoneal exudate or saline suspensions of brain, liver or spleen were used as inoculums, and inoculations were made by intracerebral, intraperitoneal and intranasal routes. Spread of infection to newborn mice through contact with infected mothers was demonstrated, but contact infection could not be established in adult mice even though contact was maintained for as long as twelve weeks.

The strain of Encephalitozoon was of low virulence, and no deaths were attributed to it. However, infected mice showed abdominal enlargement, and intraperitoneally inoculated mice killed in the relatively early stages of infection usually showed peritoneal exudate. Histologically the lesions were essentially similar in type to those noted in spontaneous infections, the most striking finding being meningoencephalitis, which was almost constantly present.

Parasites in infected animals were demonstrated in peritoneal smears and in histologic sections. The smears usually were made from peritoneal material from intraperitoneally inoculated mice, and the optimum period for finding parasites was between the tenth day and the fifth week after inoculation. By study of routine histologic sections, parasites were found in 50 per cent of the infected mice, and with special procedures they were demonstrated in a much greater proportion.

In regard to experimental infections of laboratory animals other than mice, it was found that hamsters and infant rats were susceptible, while results with a few rabbits were suggestive but inconclusive. The few attempts made to infect adult rats and guinea pigs were unsuccessful.

The parasites could not be cultivated and were not filtrable through a Berkefeld N candle. Tissues containing them remained infectious when preserved at 4 C. in either 50 per cent buffered glycerin or Tyrode's solution or when rapidly frozen or stored at — 70 C.

TOXOPLASMA AND ENCEPHALITOZOON IN SPONTANEOUS AND IN EXPERIMENTAL INFECTIONS OF ANIMALS

A COMPARATIVE STUDY

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Toxoplasmosis of man is being encountered with increasing frequency, and its recognition and diagnosis are of considerable interest. In the clinical recognition of it, the roentgen findings,[1] the ocular manifestations [2] and the results of serologic tests [3] are of great aid, but the final diagnosis depends largely on the demonstration and identification of Toxoplasma in tissue sections or in smears. The parasites may be demonstrated in sections or smears of suspected material from the patient or in animals experimentally inoculated with such material.

Considerable difficulty has been encountered in differentiating Toxoplasma from Encephalitozoon. In addition to the fact that these two types of parasites have morphologic similarities, they are both found in spontaneous, clinically inapparent infections in laboratory animals, and this may complicate animal inoculation as a diagnostic procedure.

Illustrative of the difficulty with which Toxoplasma and Encephalitozoon are differentiated are 2 cases of parasitic infection in man. Both were originally diagnosed as cases of Encephalitozoon infection but they are now generally considered to have been cases of toxoplasmosis. Torres [4a] in diagnosing a case which he observed considered both Encephalitozoon and Toxoplasma, but in a later report [4b] he favored Encephalitozoon as the etiologic agent. Levaditi [5] pointed out pathologic similarities between the lesions in Torres' case and those in experimental toxoplasmosis of rabbits, suggesting that the etiologic agent was Toxoplasma. Recent authors, including Wolf, Cowen and Paige [6] and Pinkerton and Weinman,[7] are in agreement with Levaditi. Wolf and Cowen [8] also gave consideration to both Encephalitozoon and Toxoplasma in their case, and while they diagnosed it as a case of Encephalitozoon infection, they reconsidered the case later [6] and classified it as one of toxoplasmosis.

Wolf, Cowen and Paige [6] pointed out that despite the similarities between Toxoplasma and Encephalitozoon, many of those who have worked with one or

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both of these types of organisms have not bothered to make any exact and clarifying comparison between them. Along this same line I noted recently, while reviewing the literature on Encephalitozoon infection, that the matter of differential diagnosis between Encephalitozoon and Toxoplasma had been ignored. This was particularly noteworthy since, as will be shown in the comment, the parasites described in three of these reports were probably Toxoplasma and not Encephalitozoon.

In the recent literature on toxoplasmosis there are three reports which are of special interest in regard to the differentiation of Toxoplasma and Encephalitozoon. Olafson and Monlux [9] stated that they had been unable to find criteria which would enable them to differentiate between the two types of parasites, and they suggested that the two might be identical. Wolf, Cowen and Paige [6] were of the opinion that apparent morphologic differences between Encephalitozoon and Toxoplasma were insufficient to enable one to distinguish between the two in sections and certainly could not serve as a basis for the identification of an unknown parasite as one or the other. On the other hand, they suggested that a clue to the identity of these two types of micro-organisms in tissues might be furnished by their staining reactions as well as by some pathologic features of the lesions. Pinkerton and Weinman [7] remarked on the striking resemblance of Toxoplasma to Encephalitozoon, but they listed certain differential morphologic criteria which they felt were sufficiently clear to identify a strain of parasites, which they observed in tissue sections from a patient as Toxoplasma and not Encephalitozoon.

MATERIAL STUDIED

In the division of pathology of the National Institute of Health, in Bethesda, Md., during the past few years a special effort has been made to study and compare spontaneous and experimental Toxoplasma and Encephalitozoon infections. Spontaneous infections have been encountered in normal stock animals and in animals used in various experimental studies. Experimental transmission has been done on a considerable scale and has been accompanied by pathologic studies. Some of this work has been published. A study of spontaneous toxoplasmosis in wild rats, made in association with Brigham and Pickens,[10] included the histologic changes in the brains of 10 spontaneously infected wild rats. A report of Encephalitozoon infections [11] dealt with the findings in 8 spontaneously infected laboratory animals and with various aspects of experimental transmission. The remaining data on which the comparative study is based are listed in the following pages.

Unequivocal spontaneous Toxoplasma infections were encountered in 2 guinea pigs and 1 rabbit. These animals belonged to a group of stock animals which were being deliberately examined for parasitic infection by a method combining histologic examination with animal inoculation as described in the paper on Encephalitozoon infections.[11] The spontaneous infections were found in 2 guinea pigs in a group of 17 and 1 rabbit in a group of 21.

Toxoplasma infections were found in 13 additional guinea pigs in a group of 275 during the histologic examination of brain sections in studies of experimental typhus, Rocky Mountain spotted fever and leptospirosis. The toxoplasma infections were believed to be spontaneous in spite of the fact that during the course of the experimental studies the animals had been inoculated intraperitoneally with various substances, including human blood, guinea pig blood, brain emulsions from wild and albino rats and testicular washings from guinea pigs. The clinical and gross postmortem observations (made by Brigham) were always consistent with those in the experimental infections, and no evidence of toxoplasmosis was noted. Furthermore, these animals were all killed ten to fourteen days after inoculation, and if the Toxoplasma infections had resulted from such recent inoculations, necrotizing lesions of the brain would have been likely and free parasites should have been found. On the contrary, the parasites were always grouped in large cystlike accumulations, and necrosis was not found.

Experimental toxoplasmosis was studied in mice, guinea pigs, hamsters and rats. Five strains were used for experimental transmission; two were isolated from the guinea pigs in

9. Olafson, P., and Monlux, W. S.: Cornell Vet. **32**:176, 1942.

10. Perrin, T. L.; Brigham, G. D., and Pickens, E. G.: J. Infect. Dis. **72**:91, 1943.

11. Perrin, T. L.: Arch. Path., this issue, p. 559.

which the spontaneity of infection could not be questioned, two were isolated from wild rats,[10] and the fifth was isolated from a rabbit as already noted. The two strains from the guinea pigs were dropped after the initial passage. The rat and rabbit strains were serially transmitted in mice and guinea pigs, and on several occasions single passages were made into hamsters and rats.

In all of the studies on spontaneous and experimental Toxoplasma and Encephalitozoon infections the tissues for histologic examination were fixed in a 3.7 per cent solution of formaldehyde or in Orth's fluid and routine sections were stained with Romanovsky's stain as modified by Lillie.[12] All smears were air dried, fixed in methyl alcohol and stained by Giemsa's method.

Only personal observations on the parasites and the lesions produced by them are used in the present comparative study. For observations which have already been recorded and for details which are omitted in this study, a summary of the previous reports on Encephalitozoon may be found elsewhere.[11] Wolf, Cowen and Paige [6] included in their report a bibliography on Toxoplasma, and more recent pertinent studies on toxoplasmosis have been made by Wolf, Cowen and Paige,[13] Sabin [14] and Pinkerton and Henderson.[15]

COMPARATIVE OBSERVATIONS

1. Appearance, Staining Reactions and Disposition of the Parasites.

In Histologic Sections.—(*a*) Appearance: In the comparative morphologic study of Encephalitozoon and Toxoplasma in tissue sections it was seen that both types might be represented by round, oval and elongated forms. It seemed probable that many of the round and oval forms resulted from the angular cutting of the parasites in the sections and that thus they might not represent true variations in shape. Since the elongated parasites were probably seen in their entirety, they were selected for comparison.

The elongated parasites seen in Encephalitozoon infection were definitely smaller and more uniform in size and shape than the elongated ones observed in toxoplasmosis. The former usually measured between 0.8 and 1.2 microns in width and 2.0 and 2.5 microns in length. They appeared as straight or slightly curved rods with both ends bluntly rounded; one end was usually a little larger than the other, and at times the body was slightly constricted at or near its midpoint. In contrast, the lanceolate crescentic or fusiform micro-organisms of toxoplasmosis were definitely broader and often longer than the forms seen in Encephalitozoon infection, usually measuring from 1.5 to 2.5 microns in width and from 2.5 to 3.5 microns in length.

Little or no difference could be noted in the nuclei of the two types of parasites aside from size. In appropriately stained sections apparently viable parasites of either type exhibited a single, eccentrically placed, round, oval or bandlike nucleus which, on the average, was from one-fourth to one-third the size of the corresponding parasite. In comparing the cytoplasm, however, a difference was seen. The cytoplasm of Toxoplasma often contained a few granules but it usually was more uniformly stained than the granule-free cytoplasm of Encephalitozoon, which was pale or completely unstained centrally and more deeply stained at the periphery.

(*b*) Staining Reactions: A comparative study of the staining reactions of Toxoplasma and Encephalitozoon in tissue sections revealed certain unmistakable differential points. With the modified Romanovsky stain or with hematoxylin and eosin, Toxoplasma stained much more clearly than Encephalitozoon. In tissues thus stained, Encephalitozoon was difficult to find even when the organisms were compactly arranged in sizable cystlike accumulations, while Toxoplasma stood out clearly and sharply as individual parasites or in accumulations. With the Romanovsky stain, Encephalitozoon often had a refractile, glassy appearance and was pale blue to blue gray with little or no differentiation between nucleus and cytoplasm. In contrast, the deep blue nucleus of Toxoplasma was clearly differentiated from the pink or pale blue cytoplasm. With hematoxylin and eosin, Toxoplasma was stained the same as with the modified Romanovsky stain, but Encephalitozoon did not exhibit a refractile appearance and some of the parasites were pale pink while others were pale blue.

In tissue sections stained by either of two methods which were specifically recommended for Encephalitozoon, these parasites stained more deeply than, and differentially from, Toxo-

12. Lillie, R. D.: Stain Technol. **16**:1, 1941.
13. Wolf, A.; Cowen, D., and Paige, B. H.: J. Exper. Med. **71**:187, 1940.
14. Sabin, A.: J. A. M. A. **116**:801, 1941.
15. Pinkerton, H., and Henderson, R. G.: J. A. M. A. **116**:807, 1941.

plasma, and they were easily found in sections since they were sharply contrasted with the background. With the staining method recommended by Goodpasture[16] (carbol-aniline-fuchsin, decolorized and differentiated with a 37 per cent concentration of solution of formaldehyde U. S. P. and counterstained with picric acid [trinitrophenol]), the nucleus and the cytoplasm of Encephalitozoon were a very deep blue, while the background was yellow and red-brown. In contrast, the cytoplasm of Toxoplasma was yellow and the nucleus brown, blending with the background. The method recommended by Wright and Craighead[17] (carbolfuchsin diluted 1:4, mordanted and decolorized with solution of formaldehyde, and counterstained with methylene blue) colored Encephalitozoon dark red, while the background was blue. On the other hand, the cytoplasm of Toxoplasma was pale blue and the nucleus deep blue.

Another differential staining reaction was found in the use of Weil's[18] modification of Weigert's myelin sheath stain. In brain sections stained by this method, Encephalitozoon was black while Toxoplasma was the same color as the background, a yellowish gray.

After sections were stained with steaming Ziehl's carbol fuchsin, Encephalitozoon was found to be more resistant to decolorization with acid alcohol than Toxoplasma. However, in any given section this resistance on the part of Encephalitozoon was not uniform, some groups of the parasites being pink to red and others completely decolorized.

The Giemsa stain for tissue sections had no value as a differential stain, but it colored both types of parasites deeply and clearly and brought out their internal structure, so that it was useful for comparatve morphologic study.

(*c*) Disposition: In comparing the disposition of Toxoplasma and Encephalitozoon in the tissues, it was noted that both parasites were found free, loosely grouped within cells or in compact cystlike accumulations, and these aspects of disposition were of no differential value per se. However, a clue to the identity of the parasites was gained by noting the appearance of the cystlike accumulations taken as a whole and by comparing the individual parasites in these accumulations with those that were free or loosely grouped within cells. With Toxoplasma, the outlines of the individual parasites in the cystlike accumulations were usually difficult to make out, and when this was the case each group of parasites had the appearance of a homogeneous syncytial mass in which well defined nuclei were distributed with more or less regularity. On the other hand, unless Encephalitozoon was very deeply stained, the outlines of the individual parasites in the cystlike accumulations usually could be made out; furthermore, since the cytoplasm of each parasite stained less deeply at the center than at the periphery, there was not the same homogeneity of cytoplasm as noted in the compactly aggregate parasites of Toxoplasma infection. In the comparison of the individual parasites within the cystlike accumulations with those that were free in the tissues or loosely grouped within cells, it was found that in Encephalitozoon infection the parasites maintained uniformity of size and shape in all situations. In contrast, in toxoplasmosis the parasites in the accumulations were definitely smaller and more uniform in size and shape than those seen free or within cells.

In Smears.—(*a*) Appearance: In alcohol-fixed, Giemsa-stained peritoneal smears there were striking morphologic differences in the two types of parasites. The larger size and characteristic crescentic shape of Toxoplasma readily set it apart from the smaller rod-shaped or ovoid Encephalitozoon. Toxoplasma measured from 1.2 to 3.0 microns in width and from 3.0 to 6.0 microns in length, with an average around 2.0 by 5.0 microns. Encephalitozoon averaged around 1.2 by 2.0 microns and did not measure over 2.5 microns in width and 4.0 microns in length.

The nuclei of both types of parasites were usually single, eccentrically placed and about one-fourth to one-third the size of the parasite, but in other respects they were different. The nucleus of Encephalitozoon was solid in appearance, while that of Toxoplasma was usually made up of loose strands of chromatin material. Furthermore, while the nucleus of Toxoplasma was usually round to oval, that of Encephalitozoon was more variable in shape, being crescentic, bandlike, oval or round.

(*b*) Staining Reactions: The nuclei of both types of parasites stained red to purple or dark blue, and the cytoplasm was pale blue or bluish gray. The granule-free cytoplasm of Encephalitozoon in most instances stained faintly or not at all centrally and more deeply at the periphery. In contrast, although the cytoplasm of Toxoplasma sometimes contained an occasional vacuole, it stained as deeply in central areas as peripherally and, in addition, a few small red or blue granules were often seen.

16. Goodpasture, E. W.: J. Infect. Dis. **34**:428, 1924.
17. Wright, J. H., and Craighead, E. J.: J. Exper. Med. **36**:135, 1922.
18. Weil, A.: Arch. Neurol. & Psychiat. **20**:392, 1928.

(*c*) Disposition: The two types of parasites were quite different as regards their disposition in peritoneal smears. The majority of the Toxoplasma parasites were extracellular and those that were seen within cells were most often loosely and irregularly arranged. Encephalitozoon parasites were more often found within cells, and the intracellular ones usually were compactly grouped in round or oval accumulations.

2. Clinical and Pathologic Observations.

Spontaneous Infections.—Spontaneous Encephalitozoon infection was encountered in occasional albino mice and rats and in a single guinea pig, while none was observed in a relatively small group of rabbits. On the other hand, spontaneous toxoplasmosis was encountered once in the same group of rabbits and was more frequently encountered in guinea pigs, while the albino mice and rats were not affected.

The spontaneous infection caused by either type of parasite was not recognizable clinically; all of the affected animals were apparently healthy when killed or when selected for experimental use. Furthermore, at autopsy no gross changes were noted which gave any clue to infection by either type of parasite.

In regard to the comparative histologic study of the spontaneously infected animals, no significant statements regarding visceral lesions can be made, since tissues other than brain were available for study from only a few. Furthermore, although the brain of every animal was examined, a majority of the animals with spontaneous toxoplasmosis had been experimentally infected with typhus, and the parasitic lesions of the brain were masked by this reaction. As to the remaining animals, a comparative study of the lesions of the brain did not reveal any characteristic changes which would serve to identify the infection in the absence of parasites.

Experimental Infections.—The outstanding differential clinical feature of the experimental infections caused by the two types of parasites was the greater virulence of Toxoplasma for mice. Of the three strains of Toxoplasma serially transmitted, the two isolated from the wild rats were of high virulence, while the one isolated from the rabbit was much less virulent. However, even the rabbit strain was much more virulent than the strain of Encephalitozoon; the former, after several serial passages, was lethal for mice, while the latter did not kill even after numerous serial passages.

Each strain of Toxoplasma was found to be infectious for guinea pigs, hamsters and adult and infant rats. In the relatively few attempts made to infect these animals with Encephalitozoon, hamsters and infant rats were found to be susceptible, but adult rats and guinea pigs were not.

Attempts to transmit Encephalitozoon infection to infant mice through contact with infected mothers were successful. In similar attempts with Toxoplasma the results were negative. Several attempts were made to transmit both Encephalitozoon and Toxoplasma by prolonged contact between infected and uninfected adult mice, and none were successful.

The comparative postmortem gross and microscopic observations on the experimental infections are based on the examination of mice. Grossly there were no constant or outstanding differential characteristics between the two types of infection, but certain differences were noted at times. In mice inoculated intraperitoneally with either organism, a clear or slightly cloudy viscid fluid exudate was often seen in the peritoneal cavity. In mice with severe Toxoplasma infection, however, patchy grayish areas of necrosis were usually present in the retroperitoneal tissues, while in the mice with Encephalitozoon infection they were never found. Because of the greater virulence of Toxoplasma, the mice infected with it were usually emaciated, while Encephalitozoon-infected mice were always in a good state of nutrition. Enlargement of the liver and of the spleen was more constant and usually more marked in Encephalitozoon infection than in Toxoplasma infection.

The histologic changes in Toxoplasma-infected mice showed considerable variation, depending largely on the severity of the infection; mild infections were produced by the less virulent rabbit strain, particularly in early passages, and by diluted inoculums from the rat strains.

(*a*) Brain: Meningoencephalitis was seen in mild Toxoplasma infection and in Encephalitozoon infection following inoculation by any route and was similar in most respects in the two types of infection. Granulomatous lesions with or without central necrosis were found in both, and the focal infiltrative and proliferative lesions were identical. The only differential feature, and this was inconstant, was a focal choroidal and periventricular inflammation which was occasionally seen in the mice inoculated intracerebrally with Toxoplasma but not in those with Encephalitozoon infection.

Certain brain lesions in severe Toxoplasma infections differed strikingly from any seen in Encephalitozoon-infected mice. In such infections following intracerebral inoculation of

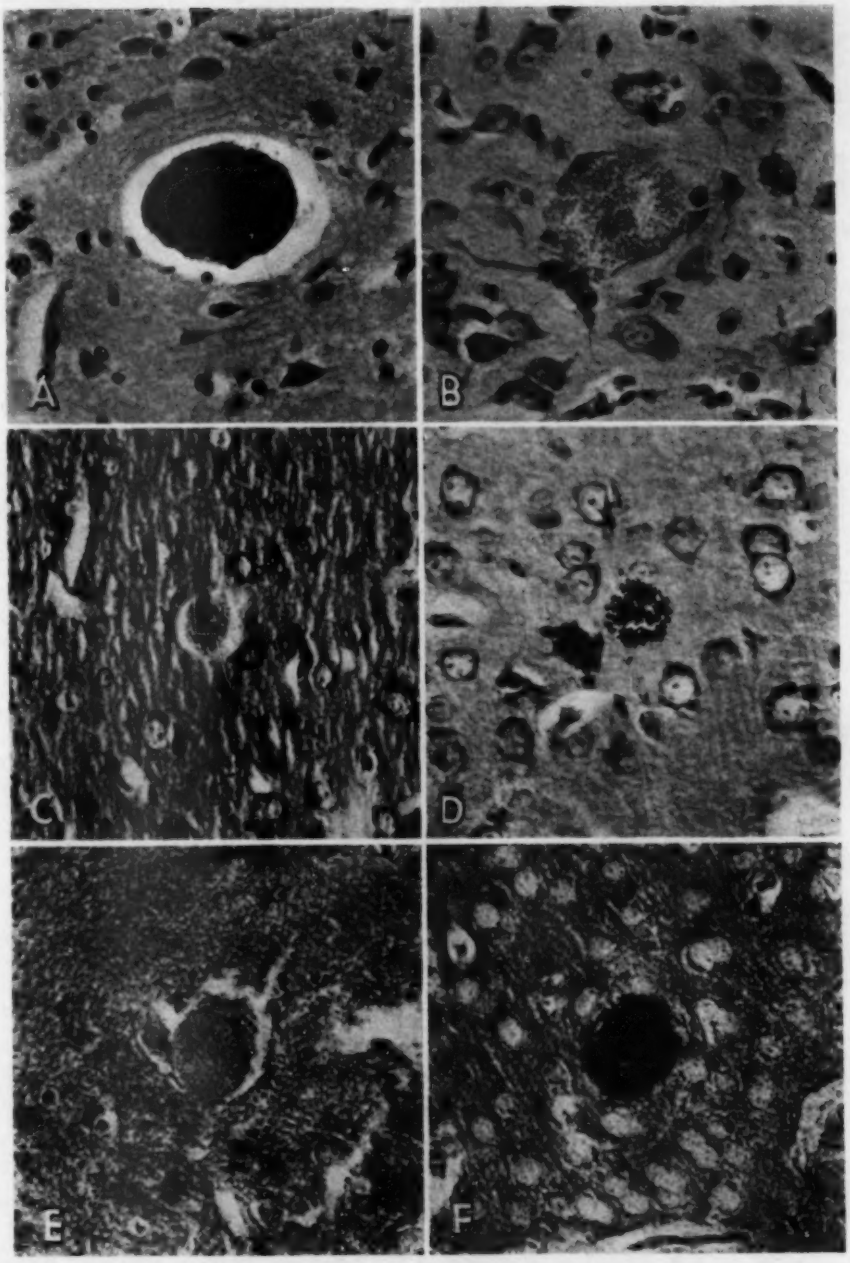

Fig. 1.—Parasitic accumulations in brain sections: *A*, Toxoplasma; *B*, Encephalitozoon; Romanovsky stain; × 375. *C*, Toxoplasma; *D*, Encephalitozoon; Wright and Craighead stain; × 375. *E*, Toxoplasma; *F*, Encephalitozoon; myelin stain; × 375.

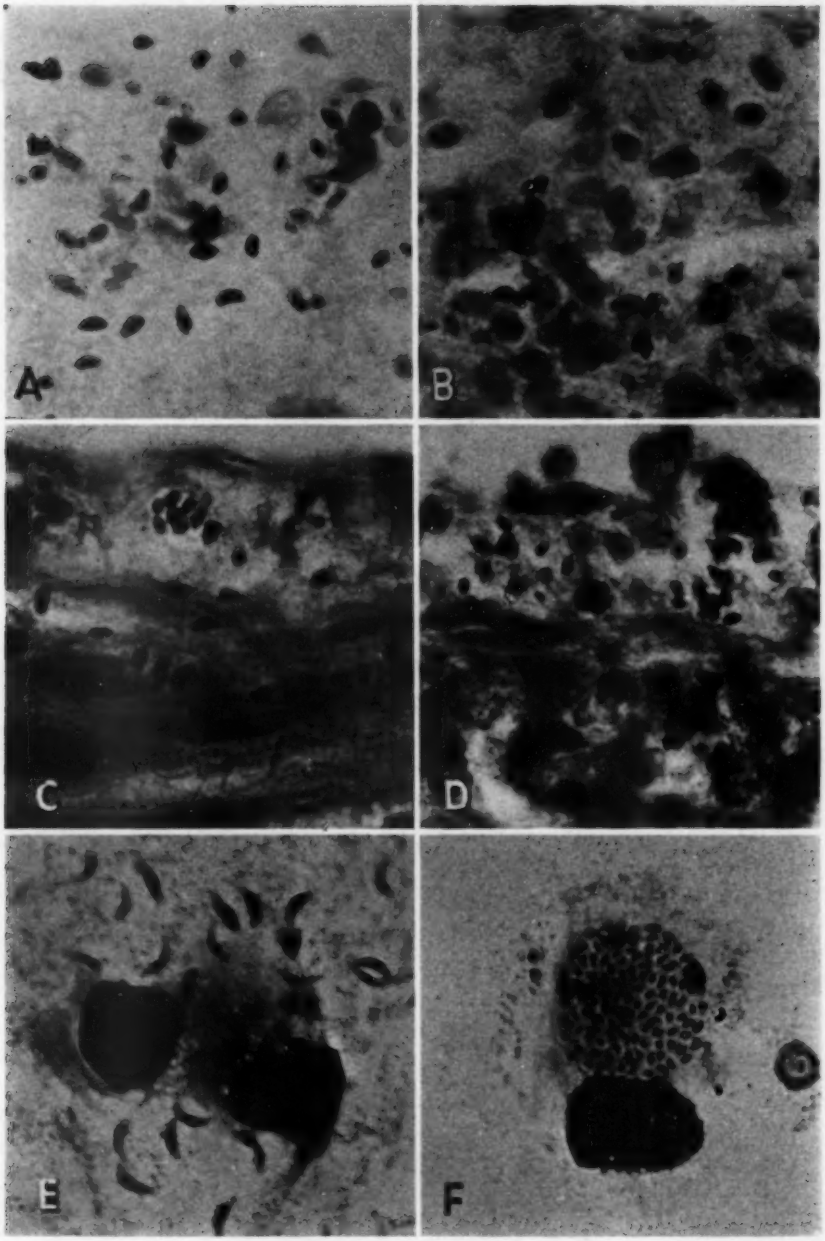

Fig. 2.—Parasitic accumulations in various tissues and in smears: *A*, Toxoplasma in brain section; Giemsa stain; × 1,900. *B*, Encephalitozoon in brain section; Goodpasture stain; × 1,900. *C*, Toxoplasma in muscularis and serosa of stomach; Romanovsky stain; × 1,900. *D*, Encephalitozoon in capsule of spleen; Wright and Craighead stain; × 1,900. *E*, Toxoplasma in peritoneal smear; Giemsa stain; × 1,900. *F*, Encephalitozoon in peritoneal smear; Giemsa stain; × 1,900.

Toxoplasma, choroidal and periventricular inflammation was constant, usually diffuse, and marked in degree, with extensive necrosis of subependymal tissue. Furthermore, in severe infections following inoculation of Toxoplasma by any route, patchy necrosis, most often cortical, was commonly seen; in intracerebrally inoculated mice the necrosis often progressed to complete disorganization of tissue, and at times there was calcification of the necrotic debris. Patchy cortical necrosis was occasionally seen in Encephalitozoon infections, but complete disorganization of tissue and calcification were not observed.

An additional differential point, in contrasting both the mild and the severe Toxoplasma infection with Encephalitozoon infection, was observed with respect to the presence of parasites within lesions of the brain. In appropriately stained sections, either parasitic strain could be found within lesions in the brain substance, but Toxoplasma was found in these areas only in the relatively early stages of infection while Encephalitozoon was found within such lesions for months after inoculation. Furthermore, in intracerebrally inoculated mice, Toxoplasma was often found in the ependymal cells of the ventricles and in the epithelium of the choroid plexuses, but Encephalitozoon was never found in these cells.

(*b*) Heart: The heart was usually involved in severe Toxoplasma infections following inoculation by any route, while in the mild infections involvement was noted only occasionally. The lesions usually found were focal infiltration and degeneration in the myocardium and focal infiltration and proliferation in the epicardium. In Encephalitozoon infections, definite myocardial lesions were not found, but epicardial changes similar to those noted in Toxoplasma infections were often seen. Toxoplasma was occasionally seen in the heart, and when present, sometimes parasitized cardiac muscle fibers and mesothelial cells of epicardium. Encephalitozoon was not seen in the heart.

(*c*) Thymus: The thymus, always unchanged in Encephalitozoon infections, was likewise unaffected in mild Toxoplasma infections. In severe Toxoplasma infections, degeneration of cortical cells was occasionally seen. Toxoplasma was found in the thymus only in association with lesions, and Encephalitozoon was never observed.

(*d*) Lungs: In Encephalitozoon infections a patchy proliferative and infiltrative pneumonic process followed intranasal inoculation but was not found after inoculation by other routes. Following the inoculation of Toxoplasma by any route, a similar pneumonic process was observed in mild infections, but in severe infections the inflammatory reaction was often more extensive, with serocellular exudation and at times focal necrosis of tissue. Focal infiltrative and proliferative pleural lesions were commonly seen in mice infected by either type of parasite after inoculation by any route. Parasites of both types were occasionally found in the mesothelial cells of the pleura, but only Toxoplasma was observed in the lungs.

(*e*) Liver: In mice infected with Encephalitozoon and Toxoplasma the findings in the liver were not particularly dissimilar except in the severe infections following intraperitoneal inoculation of Toxoplasma. In the latter, focal necrosis was commonly seen.

(*f*) Spleen: In severe infections following intraperitoneal inoculation of Toxoplasma patchy necrosis of the splenic pulp or follicles was often seen, while necrosis was never observed in Encephalitozoon disease. On the other hand, diffuse lymphoid infiltration and hyperplasia of follicles were consistently present in Encephalitozoon infections, while it was seldom encountered in toxoplasmosis. Toxoplasma was nearly always seen in lesions in the spleens with necrosis and was occasionally noted in other spleens; Encephalitozoon was rarely found.

(*g*) Mediastinal and Retroperitoneal Lymph Nodes: Except that the lymph nodes were not often found hyperplastic in Encephalitozoon infections, the changes seen in both types of infection paralleled those observed in the spleen.

(*h*) Kidneys: Focal interstitial infiltration of the kidneys was more often present and usually more marked in Encephalitozoon infections than in toxoplasmosis. Parasites of both types were occasionally encountered in the epithelium of convoluted or collecting tubules.

(*i*) Adrenal Glands: In either type of infection, small cortical foci of infiltration were occasionally observed, and at times there was associated degeneration of adjacent cortical cells. Frank cortical necrosis was encountered only in mice with severe infection after intraperitoneal inoculation with Toxoplasma. Toxoplasma was usually seen in necrotic lesions, but otherwise, like Encephalitozoon, it was rarely found.

(*j*) Stomach and Intestines: In either type of infection, lesions of the stomach and the intestines were found only in intraperitoneally inoculated mice, and primary involvement of the mucosa or the submucosa was never observed. After intraperitoneal inoculation, infiltrative and proliferative lesions of the serosa and the muscularis in mice with mild Toxoplasma

infection were essentially similar to those in mice similarly inoculated with Encephalitozoon. However, in severe Toxoplasma infections necrosis was commonly seen, while it was not observed in Encephalitozoon disease. Both types of parasites were often seen in the serosa, where they were frequently found in mesothelial cells. In the muscularis, Toxoplasma was found more often than Encephalitozoon, and while the latter was not observed in smooth muscle, Toxoplasma occasionally parasitized this type of cell.

(*k*) Uterus and Fallopian Tubes: In severely infected mice inoculated with Toxoplasma by the intraperitoneal route, primary infiltrative and necrotizing lesions were at times seen in the endometrium, the mucosa and the submucosa. Otherwise, the changes in either type of infection were similar to those noted in the stomach and the intestines.

(*l*) Ovaries and Testicles: Lesions were not found in the ovaries or the testicles in either type of infection.

(*m*) Peritoneum and Retroperitoneal Tissues: Essentially similar infiltrative and proliferative lesions were found after intraperitoneal inoculation with Encephalitozoon and in mildly infected mice similarly inoculated with Toxoplasma. In severe toxoplasmosis following intraperitoneal inoculation there was a marked inflammatory reaction characterized by abundant fibrinocellular exudate, vascular thrombosis and extensive necrosis.

3. Methods of Preserving the Parasites.

Definite differential features were noted when attempts were made to preserve the two types of parasites.

Material infected with Encephalitozoon was preserved for several weeks at 4 C. either in 50 per cent buffered glycerin or in Tyrode's solution. In contrast, Toxoplasma was similarly preserved in Tyrode's solution but not in buffered glycerin.

Material from Encephalitozoon-infected mice remained infectious after rapid freezing and storage at —70 C., while similarly treated material from Toxoplasma-infected mice did not.

COMMENT

The differential characteristics which have been pointed out in the appearance, staining reactions, disposition and preservation of Encephalitozoon and Toxoplasma are definite and unmistakable. There can be no doubt that the two types of parasites are different species, and differentiation between them should be easy on the basis of the outlined criteria.

Less definite but useful differential features have been noted in the clinical and the pathologic observations on experimentally produced infections. However, in the consideration of these differential features, certain facts should be kept in mind. First of all, it should be stressed that, except in severe toxoplasma infection, the differences were few and inconstant. Thus, as was noted in the rabbit isolated strain, Toxoplasma may be of relatively low virulence when first isolated, and at this stage differentiation from Encephalitozoon would not be possible on the basis of clinical or histologic observations alone. A second point which should be stressed is one in regard to the distribution of the respective parasites in the tissues. It should be remembered that the observations in the comparative study were based on the study of sections stained by a modified Romanovsky method; this method was much more favorable for the demonstration of Toxoplasma than for that of Encephalitozoon. Thus, some of the differences in the distribution in tissues may have been due to the relative difficulty encountered in demonstrating Encephalitozoon.

In regard to differential features noted in the clinical and the pathologic observations on spontaneously infected animals or in the susceptibility of various animals to spontaneous or experimental infection, there was not sufficient material available for study to warrant significant conclusions.

The application of the observed differential criteria to all previously reported instances of infection by Encephalitozoon or Toxoplasma was not attempted in this study. However, as already noted, the literature on Encephalitozoon infections

was reviewed for another study, and three reports were encountered in which the parasites described appeared to be Toxoplasma rather than Encephalitozoon.

Cameron and Maitland [19] classified parasites which they encountered in the brain of a rabbit as Encephalitozoon. This strain produced fatal infections in rabbits and could be serially transmitted by intracerebral inoculation of emulsions of infected brain. The parasites were clearly made out in sections stained with methylene blue and eosin, hematoxylin and eosin, or Giemsa stain, and they were found free and in cysts. The parasites in the cysts were round or ovoid and measured 1 to 2 microns in diameter, while the free ones were more variable in shape and sometimes twice as large. Microscopically, the pia and the brain were involved by an inflammatory process which included necrosis and hemorrhage; the ependyma was often desquamated, owing to inflammation just beneath it, and parasites were found in the greatest numbers in these subependymal areas. The choroid was usually involved, and parasites within it were common.

On the basis of the described findings, this strain of parasites is believed to be Toxoplasma rather than Encephalitozoon. The fact that it produced fatal infections is suggestive. The parasites stained clearly with eosin and methylene blue and hematoxylin and eosin, and it has been shown that Toxoplasma is similarly stained while Encephalitozoon is not. The parasites seen in the cysts were smaller than the free ones, and the latter were more variable in shape; these features have been pointed out as characteristic of Toxoplasma. Furthermore, since the free parasites were described as being as much as twice the size of the encysted ones, the former must have been 2 to 4 microns in diameter, which is definitely large for Encephalitozoon. Finally, the histologic observations were entirely consistent with severe Toxoplasma infection and not at all suggestive of Encephalitozoon disease; the involvement of the ependyma, the choroid and the periventricular tissues was especially significant.

Da Fano [20] made a separate report on the same strain after studying some of the material obtained from Cameron and Maitland. He described particularly the appearance and the staining reactions of the parasites, and the data he presented were basically similar to those given by Cameron and Maitland. Colored illustrations of these parasites in sections stained with hematoxylin and eosin were found in an article by Da Fano and Ingleby,[21] and the parasites bore a striking resemblance to Toxoplasma seen in similarly stained sections in the present study.

Veratti and Salla [22] also classified as Encephalitozoon parasites which they found in histologic sections prepared from the brain of a rabbit. These parasites were seen only in cystlike accumulations, and while the description of the individual parasites within the accumulations is not sufficiently detailed to permit identification, the description of the aggregations taken as a whole suggests Toxoplasma. Each aggregation was said to be made up of a protoplasmic mass containing a large number of small round masses of chromatin material, and at times, with high magnification, elliptic and tapering individual forms could be made out. In the present comparative study homogeneity of cytoplasm in cystlike accumulations characterized Toxoplasma rather than Encephalitozoon. An additional point favoring the identification of the parasites as Toxoplasma is that Veratti and Salla studied some of the preparations from Cameron and Maitland's material and were convinced that the parasites were identical.

19. Cameron, G. C., and Maitland, H. B.: J. Path. & Bact. **27**:329, 1924.
20. Da Fano, C.: J. Path. & Bact. **27**:333, 1924.
21. Da Fano, C., and Ingleby, H.: J. Path. & Bact. **27**:350, 1924.
22. Veratti, E., and Salla, G.: Boll. Soc. med.-chir., Pavia **37**:45, 1924.

SUMMARY

A comparative study of Encephalitozoon and Toxoplasma based on spontaneous and experimental infections in animals disclosed constant definite differential features in the appearance, the staining reactions and the disposition of the two types of parasites in tissue sections and in smears, and in methods by which the parasites could be preserved. Less definite but useful differential characteristics were observed in the distribution of the respective parasites in tissues and in the clinical and the pathologic observations on experimentally produced infections. On the basis of the material available for study, no significant differential features were noted in the clinical and the pathologic observations on spontaneously infected animals or in the observed susceptibility of animals to spontaneous infection.

EROSIVE (MYCOTIC) ANEURYSM OF THE HEART WITH RUPTURE

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Spontaneous rupture of the heart is not an unusual occurrence. Most commonly it is the result of a recent myocardial infarct. Among the less frequent causes of rupture of the heart are myocardial abscess, infected embolic aneurysm and erosive aneurysm. Two instances of erosive aneurysm of the heart are reported and a review of the pertinent literature is given in the following pages.

Because of the fact that the terms "embolic," "erosive" and "mycotic" are used interchangeably in the literature pertaining to aneurysm of the heart, it seems of importance to establish first a correct nomenclature. Myocardial abscesses are usually the result of pyemia and are almost always multiple. A single myocardial abscess may be caused, however, by a septic embolus and would be designated more correctly as a septic infarct. In both instances the endocardium is not primarily involved. Simple embolic aneurysms are due to lodging in small vessels of hard particles which because of their density penetrate into the vascular walls and lead to local dilatations. "Mycotic or infected embolic aneurysms" are caused by infected emboli which lodge in small vessels and produce inflammation and weakening of vascular walls so that local dilatations occur. An infected embolic aneurysm of the heart may be caused by an infected embolus attaching itself somewhere to the endocardium or lodging in a coronary artery, causing inflammation of the endocardium and myocardium, weakening of the heart wall and local dilatation. If this is the result not of an embolus but of the extension of an inflammatory process from acutely diseased cardiac valves to the endocardium or to the intima of the sinus of Valsalva with resulting ulcerative parietal endocarditis or endarteritis, respectively, and destruction of the myocardium (or media) with consequent local dilatation, the term "erosive aneurysm" is appropriate (Karsner [1]). Since, by definition, an erosive aneurysm always originates from acutely diseased cardiac valves, it may also be considered a "mycotic aneurysm."

Among 734 instances of cardiac rupture Krumbhaar and Crowell [2] and Davenport [3] mentioned abscess as the cause only five times. Benson, Hunter and Manlove [4] ascribed to the erosive aneurysm only 1 rupture in a series of 40. Not a single rupture among 46 ruptured hearts studied by Beresford and Earl [5] was caused by a mycotic or an erosive aneurysm. Willer [6] found in the literature reports of 5 cases of rupture of the heart with ulcerative endocarditis of the aortic valve and

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1. Karsner, H. T.: Human Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942, p. 392.
2. Krumbhaar, E. B., and Crowell, C.: Am. J. M. Sc. **170**:828, 1925.
3. Davenport, A. B.: Am. J. M. Sc. **176**:62, 1928.
4. Benson, R. L.; Hunter, W. C., and Manlove, C. H.: Am. J. Path. **9**:295, 1933.
5. Beresford, E. H., and Earl, C. J. C.: Quart. J. Med. **24**:55, 1930.
6. Willer, H.: Centralbl. f. allg. Path. u. path. Anat. **51**:209, 1931.

reported 1 instance observed by himself. Weiss and Wilkins [7] collected reports on 7 hearts with myocardial abscesses followed by perforation, in addition to those reported by Willer, and added 2 of their own. If rupture of the heart is defined as the opening of a cardiac cavity into the pericardium or of one cardiac cavity into another (intracardiac rupture), it seems that only 4 of their 9 hearts presented true ruptures. Each of the other 5 hearts showed perforation of a myocardial abscess into the pericardial sac, but no communication was established between the pericardial sac and a cardiac cavity.

I was able to find recorded in the literature 2 other instances of ruptured mycotic aneurysm of the heart, reported by Claytor [8] and by Nickson and Benson.[9] From the reports available it appears that the incidence of mycotic aneurysm as a cause of rupture of the heart is a little less than 1 per cent.

REPORT OF CASES

CASE 1.—A 30 year old white man had a "head cold" two weeks before admission to Michael Reese Hospital. This was followed by a sudden chill and a rise of temperature to 101 F. In the following days, the patient had repeated chills, and the temperature ranged between 101 and 102 F. On admission, he appeared acutely ill, his temperature was 104 F., there were multiple petechiae over the skin and the conjunctivas, and cervical lymph nodes were palpable. The heart was slightly enlarged; a harsh diastolic and a soft systolic murmur were heard at the apex, and a harsh diastolic murmur at the third left interspace and over the pulmonic area. The second pulmonary sound was reenforced. The fever curve was of the septic type, ranging between 101 and 104 F. The diastolic murmur increased in intensity, and the spleen became palpable. Marked cyanosis developed, and the patient died four days after admission. The clinical diagnosis was acute bacterial endocarditis involving the aortic and mitral valves.

At autopsy the heart showed the following changes: The pericardial cavity contained 150 cc. of a clear straw-colored fluid. The heart was enlarged, weighing 350 Gm. The pericardial surface was smooth and glistening and there were a few petechial hemorrhages over the left auricle. The base of the aortic leaflet of the mitral valve and the adjacent auricular endocardium were smooth and revealed a hemorrhagic zone measuring 6.0 by 14.0 mm. in the greatest dimensions. The base of the aortic valve corresponding to the region just described was covered by a number of grayish pink vegetations. The myocardium in the region of the vegetations was hemorrhagically discolored and showed many whitish gray irregular dots. The right and left aortic cusps were completely destroyed. Their bases were covered with soft friable vegetations which measured up to 12 mm. in length. Vegetations were also found in the adjacent endocardium of the interventricular septum. Between the right and left aortic cusps was a deep saccular depression covered with thrombi. This depression burrowed through the myocardium and into the endocardium of the right ventricle corresponding to the base of the pulmonary valve. In this location the endocardium was hemorrhagically discolored and distinctly bulging. The sinus of Valsalva of the right cusp of the pulmonary valve showed many grayish vegetations and an ulcerated region measuring 2.0 by 4.0 mm. in diameter. This ulcerating lesion was in direct communication with the saccular depression of the left ventricle. The remainder of the pulmonary cusps and the tricuspid valve showed no abnormalities. The myocardium on section was gray, its architecture indistinct. No abscesses were recognized. The coronary arteries exhibited a few yellow plaques of fatty degeneration.

Staphylococcus (type undetermined) was found in the valvular vegetations.

Histologic sections from the region close to the vegetations revealed many heart muscle fibers without nuclear staining. The interstitial tissue appeared widely infiltrated by polymorphonuclear leukocytes and few lymphocytes and endothelial leukocytes. Other fields disclosed well circumscribed accumulations of polymorphonuclear leukocytes, especially in the perivascular areas. There were circumscribed areas in which the myocardial fibers were pale staining and the cytoplasm granular. Sections from the vegetations revealed much fibrin and

7. Weiss, S., and Wilkins, R. W.: Am. J. M. Sc. **194**:199, 1937.

8. Claytor, A. T.: J. A. M. A. **80**:1371, 1923.

9. Nickson and Benson, cited by Benson, Hunter and Manlove.[4]

numerous red corpuscles; in other areas there were many polymorphonuclear leukocytes and fibrin in addition to clumps of bacteria.

The main diagnosis was: subacute vegetative and ulcerative endocarditis of the aortic, mitral and pulmonary valves; perforation of the left and right aortic cusps; erosive aneurysm of the conus aorticus, with perforation into the sinus of Valsalva of the pulmonary artery; insufficiency of the aortic valve; acute myocarditis; acute splenic hyperplasia; acute focal glomerulonephritis; cloudy swelling of the liver; healed pleuritis (left); bilateral bronchopneumonia; hydrothorax; hydropericardium.

Epicrisis: Following an infection of the upper respiratory tract in this patient, staphylococcic bacteremia developed, causing acute ulcerative bacterial endocarditis of the aortic and mitral valves, involving also the adjacent endocardium of the conus aorticus. From there the septic process extended through the septum and reached the pulmonic valve, producing a direct communication between the left and right ventricles (conus aorticus and sinus of Valsalva of the pulmonary artery). Thus an intracardiac rupture resulted from an erosive aneurysm.

Case 2.—A 69 year old white woman was known to have been diabetic for at least six years. Six weeks before admission to Michael Reese Hospital she first noticed an infection of her right big toe, which later progressed to ulceration and gangrene. The temperature was 99.2 F.; the pulse rate, 96; the respiratory rate, 24; the arterial blood pressure, 200 systolic and 80 diastolic. The patient appeared toxic. The skin of the back presented erythematous lesions. The lungs were normal. The heart was slightly enlarged to the left, and there was a systolic rumble at the apex. The liver was palpated 5 cm. below the costal margin. The right big toe presented moist gangrene with edema extending to the foot. The hemoglobin content was 70 per cent; the red blood corpuscles numbered 3,920,000 and the white blood corpuscles 15,600 per cubic millimeter. Blood dextrose amounted to 225 mg. and nonprotein nitrogen to 60 mg. per hundred cubic centimeters. The local condition improved following local and general treatment. About three weeks after the patient was admitted, a fever developed, the temperature rising to 102 to 103 F. Microscopic examination of the urinary sediment at that time revealed a large number of erythrocytes and polymorphonuclear leukocytes. Pulmonary consolidation and bronchial breathing developed over the lower lobe of the left lung. The patient became comatose and died five days after the beginning of the high fever.

The clinical diagnosis was: diabetes mellitus; arteriosclerotic hypertensive heart disease; gangrene of the right big toe; pneumonia of the lower lobe of the left lung.

At autopsy the heart showed the following changes: The pericardial sac contained a small amount of serohemorrhagic fluid. The upper part of the sac was partially obliterated by clotted blood which was adherent to the epicardium. The heart weighed 500 Gm. Especially the left ventricle was hypertrophic and firm. The pericardium corresponding to the upper anterolateral part of the left ventricle was covered with a soft gray-red thrombotic material, and the underlying myocardium was brown and necrotic. The chambers were moderately dilated. The tricuspid and pulmonic valves were normal. The mitral valve measured 8.2 cm. in circumference. Its chordae tendineae were thickened and partially fused, and its leaflets presented a number of gray-white glistening nodules at the line of closure. The aortic valve measured 6.8 cm. in circumference. Loosely attached to the left posterior cusp was a small mass of thrombotic brownish material which extended to the mural endocardium immediately below the valve. The cusp itself was partially necrotic and perforated in the center. The underlying myocardium was brown and necrotic. A probe was easily passed from the endocardial to the pericardial area above described. The coronary ostiums were normal; the coronary arteries were patent but markedly sclerotic, with numerous atheromatous plaques throughout. No gross evidence of thrombi or emboli was noted in these arteries.

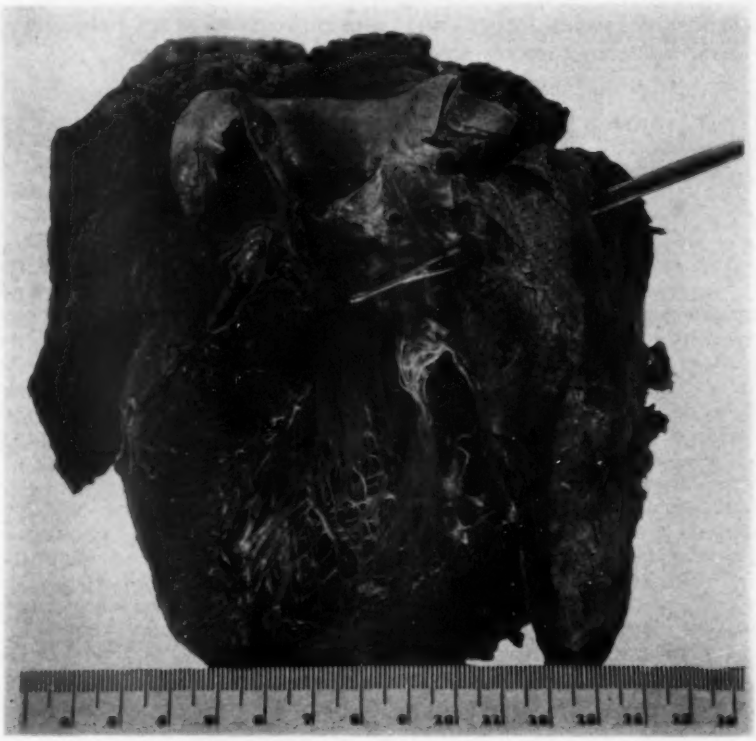
Anhemolytic streptococci, Proteus vulgaris and Staphylococcus albus were found in the ulcer of the foot; Micrococcus tetragenus, Staph. albus and anhemolytic streptococci of the cardiac vegetations.

Histologic sections through the left ventricle disclosed organizing acute pericarditis and marked increase of interstitial fibrous connective tissue. A section in the vicinity of the aneurysm revealed areas of necrosis and severe cloudy swelling. Large foci of polymorphonuclear leukocytes, lymphocytes and endothelial leukocytes were noted in the loose interstitial fibrous connective tissue. The right ventricle exhibited a marked increase of the subepicardial and interstitial fat tissue. Sections through a vegetation revealed a relatively acellular, partly hyalinized and partly necrotic tissue containing clumps of bacteria arranged in short chains,

also foci of granulation tissue with hemorrhages and infiltrations of polymorphonuclear leukocytes. Another vegetation consisted of a necrotic structure with a hemorrhagic center and many bacteria.

The main diagnosis was: severe generalized arteriosclerosis; moist gangrene of the right foot; acute ulcerative endocarditis of the aortic valve; erosive aneurysm with perforation of the heart (left ventricle); acute fibrinous pericarditis and hemopericardium; pulmonary edema; subacute intracapillary glomerulonephritis; acute splenic hyperplasia; chronic passive hyperemia of the liver and the kidneys; an old rheumatic type of endocarditis of the mitral valve and pericarditis; atrophic emphysema of the lungs; old fibrous pleural adhesions.

Epicrisis: In this 69 year old diabetic woman with generalized arteriosclerosis moist gangrene of the right foot developed. A bacteremia followed which caused acute ulcerative and vegetative endocarditis of the aortic valve with extension to



Erosive (mycotic) aneurysm of the heart with rupture (case 2). The probe lies within the perforated aneurysm. Note the vegetations on the cusps of the aortic valve.

the parietal endocardium. The mycotic process invaded the myocardium (erosive myocardial aneurysm) and the pericardium with production of acute fibrinopurulent pericarditis. Eventually perforation of the aneurysm took place with resulting hemopericardium. The thickening of the pericardium and the fibrous and fibrinous adhesions were responsible for the slow progression of the hemorrhage into the pericardial sac. This was the reason why the patient did not die suddenly.

Among the autopsy protocols of the department of pathology of Michael Reese Hospital I found 2 cases similar to the reported instances, in which, however, the erosive aneurysm did not rupture. A brief summary may be of interest.

A 20 year old white man gave a history of previous influenza and a "tired feeling." He had a prolonged loud systolic and diastolic murmur at the apex of the heart, also a palpable systolic thrill. The blood on culture yielded Staph. albus. Autopsy revealed marked hyper-

trophy and dilatation of the heart (700 Gm.), subacute ulcerative and vegetative endocarditis of the aortic and mitral valves with insufficiency of both valves, and erosive aneurysm of the conus aorticus and of the aortic leaflet of the mitral valve. The aneurysm was located

Summary of Fifteen Cases of Ruptured Embolic or Erosive Aneurysm of the Heart

Case	Author and Year	Sex	Age	Cause	Main Pathologic Observation in Heart	Site of Perforation
1	Haddon, 1884, cited by Weiss and Wilkins [7]	M	6	Embolic abscess (pyemia)	Abscess cavity in the left ventricular wall close to the coronary sinus (infected embolic aneurysm)	Left ventricle (below the coronary sinus)
2	Hart, C.: Deutsches Arch. f. klin. Med. **43**: 379, 1888	M	36	Acute ulcerative endocarditis of the aortic valve	Erosive aneurysm of the septum membranaceum and of the aorta (aortic stenosis)	Interatrial septum (intracardiac and aorta)
3	Christoph, C.: Ein Fall von doppelter Communication beider Herzhälften, Inaug. Dissert., Greifswald, J. Abel. 1897; cited by Ribbert [10b]	..	..	Acute ulcerative endocarditis	Erosive aneurysm (?) (complete data not available)	Interventricular septum (intracardiac)
4	Bönniger: Deutsche med. Wchnschr. **35**: 458, 1909	F	23	Acute ulcerative endocarditis of the aortic valve	Erosive aneurysm of the septum membranaceum	Interatrial septum (intracardiac)
5	Weise, G., cited by Willer [6]	M	25	Acute ulcerative endocarditis of the aortic valve	Erosive aneurysm of the interventricular septum	Interventricular septum (intracardiac)
6	Bayer, R.: Frankfurt. Ztschr. f. Path. **6**: 253, 1911	M	38	Acute ulcerative endocarditis of the aortic valve	Erosive dissecting aneurysm of the left ventricular wall	Left ventricle (close to the atrioventricular groove)
7	Rudolph, R. D., and Moorhouse, V. H. K.: Lancet **1**: 292, 1916	M	20	Embolic abscess (shrapnel wound of the chest)	Infected embolic dissecting aneurysm of the interventricular septum	Interventricular septum and aorta
8	Claytor,[8] 1923	M	58	Acute ulcerative endocarditis of the aortic valve	Erosive aneurysm of left atrium	Left atrium
9	Willer,[6] 1931	M	34	Acute ulcerative endocarditis of the aortic valve	Erosive aneurysm of the left ventricle	Left ventricle (close to the atrioventricular groove)
10	Benson, Hunter and Manlove,[4] 1933	F	50	Subacute bacterial endocarditis of the aortic valve	Erosive dissecting aneurysm of the interatrial septum and the left atrium	Left atrium
11	Nickson and Benson,[9] 1933	..	..	Subacute bacterial endocarditis of the tricuspid valve	Erosive aneurysm of the septum membranaceum	Interventricular septum (intracardiac)
12	Weiss and Wilkins,[7] 1937	M	73	Embolic abscess (Staph. aureus) (urinary sepsis)	Abscess of the right ventricular wall (infected embolic aneurysm)	Right ventricle (close to the atrioventricular groove)
13	Weiss and Wilkins,[7] 1937	M	78	Acute ulcerative endocarditis of the left ventricle (pneumococcus)	Erosive dissecting aneurysm of the left ventricle and atrium	Left atrium
14	Pirani, 1943	M	30	Subacute ulcerative endocarditis of the aortic valve (staphylococcus, type undetermined)	Erosive aneurysm of the interventricular septum	Interventricular septum (intracardiac)
15	Pirani, 1943	F	69	Acute ulcerative endocarditis of the aortic valve (Staph. albus and anhemolytic streptococci)	Erosive aneurysm of the left ventricular wall	Left ventricle (close to the atrioventricular groove)

immediately below the left cusp of the aortic valve. It measured 10 mm. in diameter and extended 4 mm. into the myocardium. The cavity thus formed was covered with minute vegetations and projected into the base of the pulmonary artery without perforating into this vessel. Culture from the valvular vegetations yielded Staph. albus.

This instance is similar in many respects to case 1. The septic process, however, was limited to the left ventricle of the heart, and there was no involvement of the pulmonary valve.

A 40 year old white man gave a history of a previous cold, dyspnea and high fever. He was subicteric, had rales at both pulmonary bases and a Corrigan pulse. The heart presented a loud systolic murmur at the apex and a long diastolic murmur at the base. The electrocardiogram revealed first degree atrioventricular block and left ventricular preponderance. The blood cultures were negative. Autopsy disclosed acute ulcerative and vegetative bacterial endocarditis involving the aortic valve, with perforation of the posterior and left cusps and extension into the mural endocardium and myocardium of the left ventricle and left atrium and into the pericardium. Directly adjacent to the left cusp of the aortic valve, within the left auricular and ventricular wall was an abscess measuring 1.3 by 0.9 cm. in greatest dimension, containing thick purulent material and presenting a definite pyogenic membrane. The abscess was bulging into the left auricular cavity, with discoloration of the endocardium. Septic infarction of the anterior papillary muscle and acute fibrinosanguinous pericarditis were also present. Cultures of material from the valves yielded streptococci (type unidentified) and Bacillus coli.

In this particular instance the erosive aneurysm of the heart was close to rupturing either into the auricular cavity or into the pericardial sac. The patient went downhill gradually and died with symptoms of heart failure.

The accompanying table contains a short summary of 15 instances of ruptured mycotic embolic or erosive aneurysm of the heart, 13 collected from the literature and the 2 now described. It may be noted that among these 15 instances there are only 3 of embolic aneurysm, the remaining 12 being cases of true erosive aneurysm.

COMMENT

From the table it would seem that ruptured infected embolic and erosive aneurysms of the heart occur more frequently in males than in females, with a ratio of 5 to 1. This confirms previous observations of Willer [6] and of Weiss and Wilkins [7] but cannot be satisfactorily explained. The average age of the 13 patients considered here was 41 years, with the aneurysms occurring more frequently in the third and fourth decade of life.

Acute ulcerative endocarditis was encountered nine times and subacute bacterial endocarditis three times. Of the two types of endocarditis, the first is therefore the more frequent cause of erosive aneurysm and of other types of mycotic aneurysm of the heart. Among 15 cases infected embolic aneurysm was present in only 3 (cases 1, 7 and 12); in all 3 instances it originated from an extracardiac disease on the basis of a generalized sepsis. This small group presented a variation of age much more marked than that found in the cases of erosive aneurysm.

Occasionally, acute vegetative and ulcerative endocarditis may be considered as explaining coronary embolism and myocardial abscess (infected infarct). It is unlikely, however, that endocarditis may cause rupture of the heart through an embolic mechanism. In all cases reported here the extension of the inflammatory process by continuity from the valvular apparatus to the parietal endocardium and to the underlying myocardium was the only factor that could be considered responsible for the perforation of the heart, and neither gross nor histologic evidence of coronary embolism was present. Thus true infected embolic aneurysm of the heart is exceedingly rare. When bacteriologic studies were done, the cultures yielded hemolytic and anhemolytic streptococci, staphylococci and pneumococci.

A pericardial type of rupture was present in 7 instances, an intracardiac type in 6, while pericardial and intracardiac types were present together in 2 other cases. The anterior wall of the left ventricle in the region of the atrioventricular groove and the septum membranaceum were by far the most frequent sites of rupture. The left atrium and the right ventricle were found to be the sites of rupture in 2 and

1 case, respectively. This agrees with the statement of Weiss and Wilkins that abscesses have a certain predilection for the fat tissue at the junction of the atria with the ventricles.

The mechanism of the formation and rupture of a mycotic aneurysm was well described especially by earlier authors. In acute ulcerative endocarditis, if the septic process spreads from the affected valve to the parietal endocardium, this may become necrotized by the bacteria. Later it will be excavated by the impact of the blood stream with eventual production of an aneurysm. This is nothing more than a true acute ulcer which because of the necrosis and inflammation of the surrounding myocardium may subsequently perforate. Anatomically, the base of this ulcer consists of necrotic muscle fibers and material derived from the blood stream (fibrin, leukocytes, red blood corpuscles). Before perforation takes place the wall may bulge either into the pericardial sac or into one of the cardiac chambers with formation of a saccular aneurysm.[10] According to Kauffman [11] and Rokitansky,[12] the chances of intracardiac rupture of the heart are particularly great when the endocardiac process involves the septum membranaceum, which then bulges toward the opposite chambers of the heart or toward the pulmonary artery. In spite of the formation of a mural thrombus, perforation of the septum is most likely to occur. It is clear that most of the ruptures occur, or at least originate, in the left ventricle, as bacterial endocarditis is far more common in the left side of the heart.

In subacute bacterial endocarditis the inflammatory process of the aortic and rarely of the tricuspid and mitral valves may extend into the subjacent myocardium of the interventricular septum or into the membranous septum. In contrast to the occurrence of this complication in acute bacterial endocarditis, lesions of this type, according to Libman,[13] almost never cause interventricular perforation. In some cases, however, as a complication of the valvular endocarditis an erosive aneurysm may form in the sinus of Valsalva or in the valve itself. From here in rare instances the infection may diffuse through the aortic myocardial juncture to the wall of the ventricle or of the atrium with formation of a long and tortuous tract. Thus a dissecting aneurysm of the heart ensues, the origin of which was in the aorta. The fistulous tract may end in an abscess cavity within the cardiac wall, as was observed by Pepere,[14] or more commonly may reopen into one of the cardiac chambers or into the pericardial sac. Less frequently the dissecting mycotic aneurysm originates either from parietal endocarditis or from septic embolism of a branch of a coronary artery (case 7).[15]

Four of the 15 instances collected here (cases 6, 7, 10 and 13) can be considered instances of dissecting mycotic aneurysm either because of the length and tortuosity of the tract within the cardiac wall or because of the origin from a mycotic aneurysm of the ascending aorta. In other instances, as in case 14, the formation of a large

10. (*a*) Ponfick: Virchows Arch. f. path. Anat. **58**:528, 1873. (*b*) Ribbert, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 21, p. 228. (*c*) Reinke: Berl. klin. Wchnschr. **49**:1585, 1912. (*d*) Bain, C. W. C., and Wray, S.: Brit. Heart J. **3**:132, 1941.

11. Kaufmann, E.: Lehrbuch der speziellen pathologische Anatomie, ed. 9, Berlin, W. de Gruyter & Co., 1931, vol. 1, pp. 29 and 58.

12. von Rokitansky, C.: Lehrbuch der pathologischen Anatomie, Vienna, W. Braumüller, 1855-1861, vol. 2, p. 275.

13. Libman, E.: Subacute Bacterial Endocarditis, New York, Oxford University Press, 1941, p. 16.

14. Pepere, A.: Arch. per le sc. med. **33**:515, 1909.

15. Glass, G.: Frankfurt. Ztschr. f. Path. **11**:428, 1912. Vestberg, A.: Nord. med. Ark. **29**:26, 1897. Benson, R. L.: Am. J. Path. **2**:826, 1926.

abscess had caused rupture of the cardiac wall at some distance from the origin of the mycotic process without forming, however, a dissecting type of aneurysm.

A similar dissecting mycotic aneurysm has been described in the aorta as a rare complication of bacterial endocarditis of the aortic valve. In an interesting case recently reported by Bartol, Edwards and Lamb [16] a mycotic dissecting aneurysm of the aorta was associated with a mycotic aneurysm of the heart in the region of the right auricle which was apparently very close to rupture into the pericardial sac.

Ponfick [10a] noted that often the cardiac wall behaves like that of an artery and the pericardium like the arterial adventitia, so that the thickening of the pericardium protects the aneurysm and the rupture of the heart may be a slow one, taking days or weeks to develop. This "slow rupture" is obviously due to a slow leak of blood into the pericardial sac through one or many small openings and its successive clotting in layers on the surface of the heart. The formation of thrombi in the aneurysmal sac or over the mural endocardium and the frequent presence of old or recent pericardial adhesions contribute to the prevention of a large immediately lethal extravasation of blood.[17]

The very frequent location of the rupture in the anterior wall of the left ventricle close to the atrioventricular groove, in my opinion, is due to the particular structure of the myocardial-aortic juncture and to the presence of abundant subepicardial fat, which makes this region a point of least resistance in the cardiac wall. Here the aseptic process may more easily extend to the myocardial tissue near its origin at the aortic ring, following the loose fibrous connective tissue around the large branches of the left coronary artery, and thus reach the pericardium. The presence of a pericardial wedge in this region and the proximity of the aortic ring to the pericardium make it possible for an infection originating in the former to pass easily by contiguity into the pericardium and vice versa (Gross and Kugel [17b]).

It is also possible that the direction and the pressure of the blood stream in the region of the conus aorticus further contribute to the excavation and eventual rupture of an endocardial ulcer in that region more than in any other area of the cardiac wall.

SUMMARY

There are two types of mycotic aneurysm of the heart: infected embolic and erosive. Mycotic aneurysm of the cardiac wall is a relatively rare but distinct pathologic entity and may be the cause of rupture of the heart, accounting for less than 1 per cent of all instances of rupture. Of 15 reported cases of rupture, the rupture was on the basis of an acute or subacute bacterial endocarditis with formation of an erosive aneurysm in 12, while in the other 3 it was caused by an infected embolic aneurysm.

Rupture of the heart is relatively more frequent in mycotic than in any other single group of cardiac aneurysms. The locations of the cardiac ruptures are in order of frequency: the left ventricular wall anteriorly in the region of the atrioventricular groove, the interventricular septum and the interatrial septum.

Mycotic aneurysm of the heart may be saccular or dissecting, the first type being much the more frequent.

16. Bartol, M. G.; Edwards, J. E., and Lamb, M. E.: Arch. Path. **35**:284, 1941.

17. (*a*) Silverthorn, G.: Canad. Pract. **23**:193, 1898. (*b*) Gross, L., and Kugel, M. A.: Am. J. Path. **7**:445, 1931.

MECHANISM OF RELEASE OF COLLOID

AND THE SIGNIFICANCE OF THE SPECIFIC CRYSTALLINE SUBSTANCE DEMONSTRATED IN THE THYROID GLAND HISTOLOGICALLY

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These studies were made to test the applicability to the thyroid gland of the concept and methods outlined by me in a recent publication on the epithelial functional rejuvenation of certain cells in the gastrointestinal tract.[1]

It was realized from the beginning of this work that in the case of the thyroid gland one is faced with a number of difficulties and complications. Unlike the other glands of internal secretion, the thyroid gland stores its secretion in the follicles, and the discharge of the follicular content into the circulation is an independent process and is not synchronous with the production and secretion of that content into the follicles. This mechanism precludes the necessity of continuous secretion by the cells of the gland, and it is therefore difficult to evaluate either the rhythm of secretion or the effect of tear and wear on the individual cells of the epithelial lining. Furthermore, there remain other unsolved problems which make difficult an intelligent interpretation of the processes of the thyroid gland. Every pathologist knows that the histologic state of the thyroid gland is in many instances fully unreliable when one attempts to explain the clinical signs of thyroid disease. On the other hand, there are two facts which remain unexplained by biochemists. One is that certain samples of desiccated thyroid gland yield no crystalline thyroxin although they are highly potent physiologically. The second fact is that desiccated or fresh thyroglobulin may produce more calorigenic effect than does the thyroxin contained therein. To add to the difficulties, one is faced, furthermore, with such unsolved problems as (1) the process by which an active hormone is formed, mobilized and thrown into the circulation; (2) the ways in which the active and the inspissated colloids are removed from the follicles; (3) the mode of formation and the significance of the peripheral and central vacuoles that are demonstrated in histologic preparations; (4) the tinctorial peculiarities of the colloid; (5) the significance of the intracellular accumulation of lipoids, and (6) the nature of the so-called postoperative storm.

In all fairness it must be admitted that morphologic science has contributed the least to knowledge of the thyroid gland. The reason for this is that the available methods are limited in scope and are inadequate to offer any new knowledge. In these studies efforts were made to evolve new methods and to reexamine some of the aforementioned unsolved problems with the aid of these methods.

MATERIAL AND METHODS

The material used consisted of 62 human thyroid glands obtained surgically and the thyroid glands of 50 rabbits and 18 guinea pigs. The human material included all varieties of toxic and nontoxic glands and proliferative types of adenomatous goiter.

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1. Popoff, N. W.: Arch. Path. **27**:841, 1939.

Both the rabbits and the guinea pigs were kept in sunny, airy quarters and fed a well balanced diet with an abundance of fresh greens throughout the entire period of the investigation. The majority of the rabbits examined were employed for an ovulation test; 15 gave a positive and 25 a negative result in that test. The guinea pigs were normal in every way. Seven of them were examined at various periods of gestation. The animals were killed in a uniform fashion by air embolism. The thyroid gland was placed in the fixator within fifteen minutes after being cut in two longitudinally.

Human thyroid glands were obtained immediately after their removal, cut into pieces not thicker than 3 mm. and placed without delay in the fixator. The record of each case was supplemented by a complete clinical history. Thyroid glands from postmortem material were considered as unreliable and were not included in this report. Helly fixator was used as a control in every case, and the time of fixation was twenty-four hours. Three other variants of fixation were used in this investigation. The first variant was carried out in the following manner: Tissue was placed for twenty-four hours in a solution of 45 per cent formaldehyde, 30 per cent acetone and 1.5 per cent Grübler's water-soluble safranin in equal parts. The tissue was then rinsed in distilled water and placed for twenty-four hours in a solution of 4 per cent acetic acid and 3 per cent zinc sulfate in equal parts. The tissue was then washed in several changes of distilled water for twenty-four hours, dehydrated in alcohol, cleared in chloroform and embedded in paraffin. In the second variant of fixation tissue was placed for twenty-four hours in a solution made of 45 per cent formaldehyde, 30 per cent acetone and 1.5 per cent Grübler's toluidine blue in equal parts. The tissue was then rinsed in distilled water and placed for twenty-four hours in an acetic acid–zinc sulfate solution as in the first variant of fixation. After being washed for twenty-four hours in several changes of distilled water, the tissue was dehydrated and embedded in paraffin in the usual manner. In the third variant of fixation tissue was handled as in the first variant and then, following washing in distilled water after having been in the acetic acid–zinc sulfate solution, it was placed for twenty-four hours in 3 per cent potassium bichromate, washed in distilled water for twenty-four hours and embedded in paraffin in the usual manner. The rationale of such methods of fixation will be considered in the section headed "Comment." A number of supplementary procedures and control tests will also be discussed later. In the course of these studies the serial section method was used.

Sections from the material fixed in Helly's fluid [2] were stained with potassium bichromate–eosin–silver nitrate–methyl blue method (method 2) published elsewhere,[1] slightly modified. The modification involves the preparation of a reducer. Grübler's water-soluble aniline blue could not be secured and was replaced with Grübler's methyl blue, which was used in 1 per cent solution. In preparation of the reducer 1 drop of hydrazine hydrate is added to 2 cc. of methyl blue. The sections made from the material fixed with the first and second variants were deparaffinized with the aid of xylene and thereafter no supplementary staining was needed. Besides saving labor, this permits preparation of any number of uninterrupted serial sections from each block. Sections made from the material fixed with the third variant of fixation were stained in addition with my potassium bichromate–eosin–silver nitrate–methyl blue method.

RESULTS

The main interest has been centered around the human thyroid gland, and the observations on it will be reported first. Staining method 2 applied to the material fixed with Helly's fluid is of considerable interest. It gives an excellent impregnation of argyrophilic reticulum. It permits tinctorial differentiation between old, centrally located and fresh, peripherally located colloid. The old colloid is stained red rose with eosin, and the fresh colloid is stained bluish green or olive green with methyl blue. As both the dyes employed are acid dyes, the terms "acidophilic" and "basophilic" are not proper ones to use. This tinctorial phenomenon considered alone and without other concomitant cytologic features is of little physiologic or pathologic significance. It is conditioned by the physical state of the colloid and can be easily destroyed. Freezing of fresh thyroid tissue with solid carbon dioxide for five minutes destroys completely the specific tinctorial affinity of

2. Helly's fluid is Zenker's solution prepared with neutral solution of formaldehyde U. S. P. instead of glacial acetic acid.

the colloid, which after this procedure appears dirty greenish and without a trace of red staining. In addition to red staining the old colloid shows, particularly in a quiescent gland, a silver-reducing amorphous precipitate.

Material fixed with Helly's fluid, and especially that from a functionally active gland, shows a great deal of peripheral vacuolation of the colloid. The peripheral vacuoles contain no stainable or silver-reducing matter. This method of fixation causes also considerable shrinking and peripheral retraction of the colloid. Some follicles show central vacuoles, which are usually solitary and of considerable size. The number of follicles containing such central vacuoles varies from gland to gland. No cells or cellular debris of any kind has been found in these central vacuoles as examined in serial sections. The vacuoles show, however, a fine or a coarse granular matter, in the shape of a globe, which stains yellow, orange, brown-orange or black. In an occasional follicle such a globe shows single or multiple hernia-like protrusions directed externally toward the epithelial lining of the follicle. Gradually these herniations reach the epithelial lining, and the cells which enter in contact with the distal ends of the protrusions begin to take up the content of the protrusions. The cells engaged in the absorption of content appear at first homogeneous yellow, then finely granular and orange, and later on they are loaded throughout with coarse granular orange-black matter (fig. 1 *A*). This phenomenon has been observed in a number of glands. The manifestations of it vary, and they may even vary in the same gland. One gland may show only a few follicles with central yellow-stained globes and without signs of herniation. Another gland may show a number of follicles with central globes in various phases of herniation and actual absorption of the content of the herniated portions by the epithelial cells. In certain glands, particularly in senile ones after the age of 60, a great number of follicles show centrally located globes of small size which have a dark orange or black appearance and which show no signs of herniation and no absorption of their content by the epithelial cells. These observations make it clear that the human thyroid gland possesses a special mechanism by which it is able to remove the central inspissated colloid and to use it over again. With advancement in age, the mechanism becomes ineffective, and the gland acquires a specific morphologic appearance. Additional evidence in support of this concept will be presented in succeeding pages.

The following additional observations of significance were disclosed with the aid of staining method 2 and are the next point of presentation. Examination of hundreds of blocks from various thyroid glands has shown that this method in many instances demonstrates clearly the presence of intercellular spaces, which appear like minute canaliculi impregnated with silver. In some cases these canaliculi are numerous and are found in all the blocks of the same thyroid gland. In other cases such canaliculi are few and are found in spots here and there, and in certain cases no canaliculi are found in the sections of all the blocks examined. Whenever they are numerous and conspicuous, they are shaped like minute tubules, each with a wide basal and a tapered, narrow peripheral end (fig. 1 *B*). Occasionally they show a rose or yellowish content. At no time are such canaliculi found in empty follicles. There is a strong morphologic reason to believe that these canaliculi represent a special system which is connected with the release of follicular content into the circulation. Observations in cases of adenoma of the thyroid gland offer particularly convincing evidence in support of such an assumption. As compared with the normal gland the gland showing this type of goiter is lacking in structural perfection. Here, both the vascular and the interalveolar ground structures are improper and defective. Furthermore, one may see here

all the phases of progressive organoid differentiation, which may, at the same time, show signs of structural and functional maladjustment with consequent involution and regressive changes. In these cases the most apparent difficulty is with the release of colloid. Structurally defective areas of such goiters frequently show widely distended canaliculi with transformation of their basal portions into large cystic formations (fig. 1 *C*). In some instances cystic distention of the canaliculi, especially of their basal parts, leads to their rupture with consequent escape of

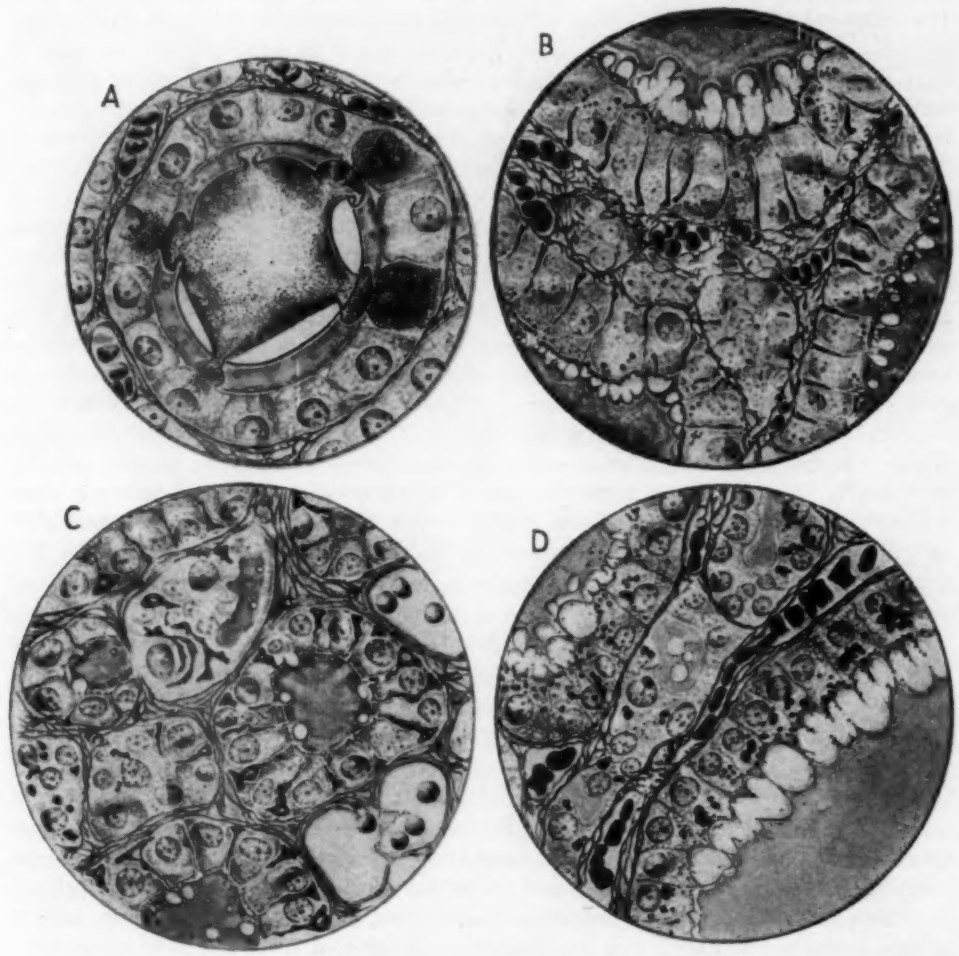

Fig. 1.—*A*, section of a single follicle demonstrating intracellular reabsorption of central inspissated colloid. Note the herniation-like protrusions directed toward the acinous epithelium, which shows absorption of the hernial content. × 512. All the illustrations of this article were made with the aid of a camera lucida and from human thyroid glands. *B*, section demonstrating intercellular silvered canaliculi through which the active colloid is released from the follicles. × 512. *C*, section demonstrating distention of the basal parts of intercellular canaliculi caused by interference with the release of colloid. × 512. *D*, section demonstrating lipoid granules in the acinus epithelium. × 512.

content and detachment and desquamation of the adjacent epithelial lining. It is therefore possible with this method to determine whether the gland examined is in a phase of colloid release and whether the release is normal or abnormal.

The next point of reporting concerns certain cytologic observations which offer additional information needed for the clarification of the problem under discussion. Depending on the functional state, the acinous epithelium may be low cuboidal, high cuboidal or columnar. In some instances one side of the follicle may be lined with a low cuboidal epithelium whereas the cells of the opposite side are tall. The low cuboidal cells of the so-called quiescent gland are of two types. One type has a more or less compact bluish nucleus, an almost colorless cytoplasm and no inclusions. The other has a ruby red or dark brown nucleus and a cytoplasm filled up with bulky argentaffin granules. This difference is shown particularly well after fixation with Zenker's fluid, but these two types of low cuboidal epithelium are indistinguishable in sections stained with hematoxylin and eosin. The latter type of epithelium is most commonly found in large, distended acini filled to capacity with colloid. This cytologic peculiarity, together with the absence of intercellular canaliculi, is apparently responsible for the difficulty with the release of colloid.

In a number of cases, especially in those in which the gland shows no intercellular canaliculi, the epithelial cells show apical minute round granules. Some of the granules are red brown; others show silver impregnation of varying intensity. They are found in certain types of adenoma, in thyroid glands without adenoma and even in so-called Sanderson's polsters lined with high columnar epithelium (fig. 2 *A* and *B*). There is every reason to believe that these apical inclusions are secretory granules. They differ from other frequently found silver-impregnated granules, which are dispersed in the cytoplasm, have a bulky appearance and show all the characteristics of a waste pigment of lipoid nature (fig. 1 *D*). It is worthy of note that these inclusions do not interfere with the release of colloid, as they are found infrequently in the presence of intercellular canaliculi. Moreover they are demonstrated in sections stained with hematoxylin and eosin. A consideration of intracellular inclusions would be incomplete without mention of another type of argentaffin granules. These are found mostly along the basal portion of acinous epithelium. These granules are characterized by their resistance to various chemical and physical factors. The cells which contain them resemble closely the argentaffin cells of the intestines. Their number varies, and they are less frequent in the thyroid gland than in the intestinal tract. In my opinion these cells are identical with the argentaffin cells of the gastrointestinal tract in regard to their mode of formation and functional significance.[1]

The results obtained with the aid of the aforementioned three variants of fixation are of considerable interest. Cytologically speaking, these methods are inferior to fixation with Helly's fluid, but in many other ways they are more instructive. Unlike the use of Helly's fluid, they do not cause shrinking and retraction of colloid. The dye is a part of the fixator, and therefore the sections do not require additional staining. Both the safranin and the toluidine blue give good nuclear staining, and the staining of the cytoplasm and the ground substance is adequate for general topographic orientation. The most striking and constant microscopic observation is that the peripheral vacuoles are rare and few, while in the material fixed in Helly's fluid they are numerous. Apart from the fact that the vacuoles are less numerous, their number varies considerably from gland to gland. In the beginning it was difficult to account for these numerical variations. Later it was found that the duration of operation and unavoidable delays in fixation were apparently responsible for these numerical variations. The longer the delay the more numerous were the vacuoles. In some instances, however, in spite of delay in fixation the peripheral vacuoles were rare or were

not found at all. In order to obtain more definite information tissues were fixed after delay of two, six, twelve, eighteen and twenty-four hours, during which time tissues were kept in a moist chamber at room temperature. The results showed that delay in fixation was actually responsible for the increase in number of the peripheral vacuoles. Here again exceptions were found, as certain samples of

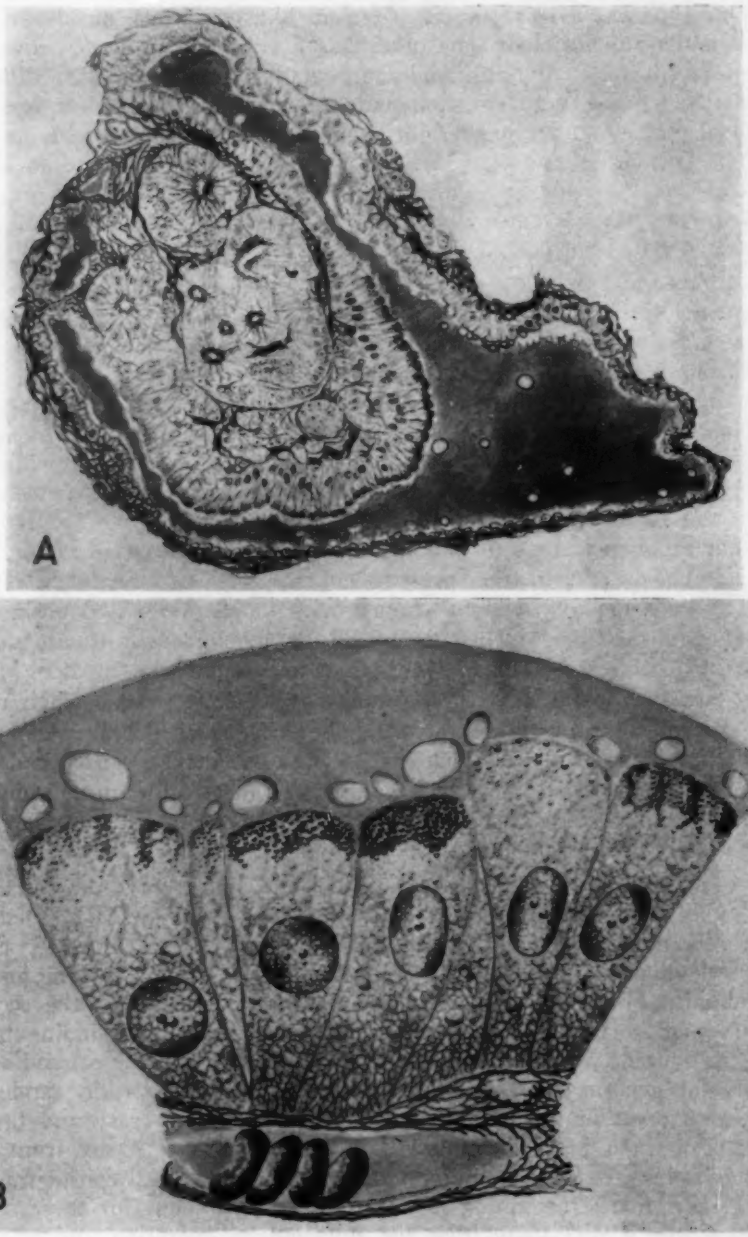

Fig. 2.—*A*, section demonstrating apical secretion granules in the high columnar epithelium of a Sanderson polster protruding into the follicular cavity. × 111. *B*, section demonstrating apical secretion granules under high magnification. × 891.

thyroid tissue in spite of delay in fixation failed to show increase in the number of vacuoles. A further step was to investigate the effect of freezing for ten minutes with solid carbon dioxide preliminary to fixation. Tissues so treated showed either no peripheral vacuoles or only a few as compared with corresponding control preparations made without freezing, which contained large numbers.

Another unusual observation is that almost every vacuole contains a crystalline substance. This substance appears in the form of stars, thorns or bundles of straight or slightly curved needles, and it is almost always located at the periphery of the vacuole (fig. 3*A*). In some instances vacuoles are not found, but there are here and there in the follicle minute crystals and imperfectly crystallized matter. This finding is particularly conspicuous in material subjected to freezing before fixation. In still other cases the colloid is found to be free of any crystalline substance, and at the same time not a single vacuole is found in serial sections covering the entire follicle. It is apparent that the formation of vacuoles is closely associated with the presence or the absence of crystalline substance and that vacuolation or liquefaction of colloid represents some kind of enzymatic digestion. The crystalline substance therefore has to be considered either as a product of digestion or as a causative factor of digestion. Further studies offered additional evidence in support of the assumption that the crystalline matter is a causative factor of digestion. In certain thyroid glands and even in certain blocks of the same gland there is found a crystalline substance in the apical parts of the epithelial cells. As a rule this substance is never found in the basal parts of the epithelial cells. These apical crystals are much smaller than the intravacuolar crystals, and they appear like torch-thistles with their free thorns directed toward the follicular cavity (fig. 3*B*). This topographic peculiarity, together with the close similarity between apical and intrafollicular crystals, led to the assumption that the crystalline matter in question is a product of epithelial secretion. This method also demonstrates intrafollicular central solitary vacuoles, which have already been described in connection with the findings following fixation with Helly's fluid. They also contain a crystalline matter, which is found only on the periphery of the vacuole and which shows less perfect crystallization. It is significant that in contrast to the occurrence of peripheral vacuoles that of central vacuoles is unaffected by delay in fixation or by freezing preliminary to fixation. Putting together all the hitherto reported observations concerning the vacuoles, one finds it reasonable to conclude in regard to their formation that the central vacuoles are intravital and that the peripheral vacuoles are supravital.

The application of dilute solution of formaldehyde–acetone–toluidine blue (second variant of fixation) served to corroborate the previously reported observations. Just as with the first variant of fixation, sections do not require additional staining. With this fixator, sections show peripheral vacuoles which contain not crystalline but coarse amorphous particles, which are always found at the periphery of the vacuole, and with each vacuole containing two, three or more dark blue particles (fig. 3*C*). Similar particles are found from time to time in apical parts of acinous epithelium. The central solitary intrafollicular vacuoles show rather coarse, sometimes rectangular particles, located always at the periphery of the vacuole (fig. 3*D*). Incorporation of safranin or toluidine blue in the fixator is essential for the precipitation and demonstration of the crystalline substance and amorphous particles, which are considered as identical in nature. This substance is shown in both the frozen sections and the sections from the paraffin-embedded material. It is insoluble in alcohol, acetone, chloroform and acid but is soluble in ammonium hydroxide and in sodium phosphate. It shows silver

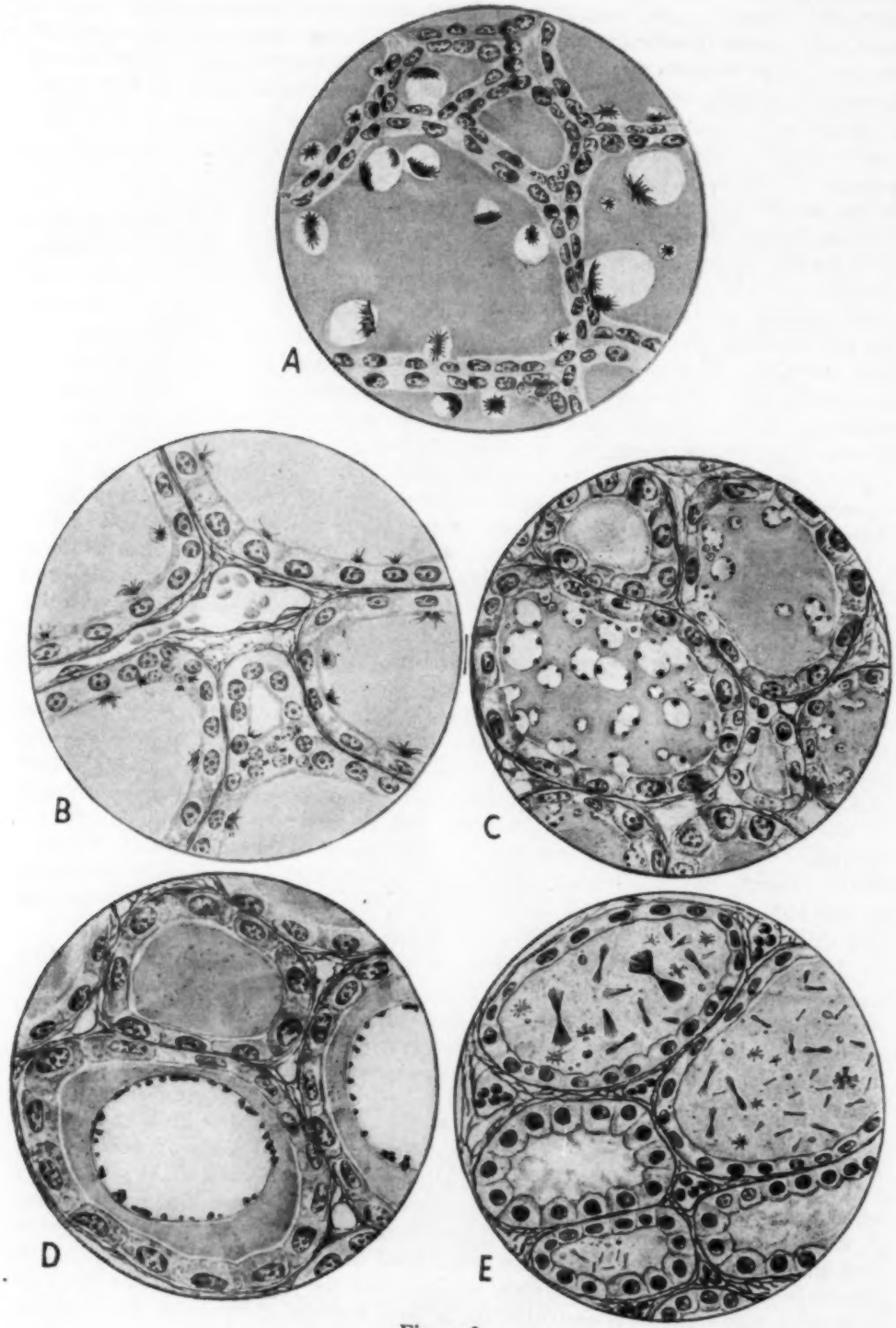

Figure 3

(See legend on opposite page)

reduction of varying intensity. Left for hours in distilled water, it remains unaltered, but if, after prolonged washing in distilled water, the slide is put into a neutral solution of peptone it disappears within ten hours and neither restaining with safranin or toluidine blue nor silver reduction discloses any traces of it. The melting point of this substance is around 210 C. It has been mentioned that as a rule the substance is found in the colloid and in the apical parts of the acinous epithelium, and only in rare instances is it seen in the capillaries and postcapillaries. It is to be noted as important, however, that a similarly stained but morphologically different substance is found from time to time outside the acini and in blood vessels of all sizes. It differs from the aforementioned crystalline substance in its shape and in its insolubility in ammonium hydroxide. Furthermore, such a substance is found in other organs and tissues, and comparative studies suggest that it is either a mucoitin polysulfuric ester (heparin) or a substance close to hyaluronic or chondroitin-sulfuric acid. It is worthy of mention here that fixation variant 2 is the easiest and most reliable method for the demonstration of mast cells, which are strikingly rare in the thyroid gland and which at present are considered as being related to the production of heparin.

The observations made with the aid of the third variant of fixation are of considerable interest. Sections from the material prepared with this fixator require additional staining by the potassium bichromate–eosin–silver nitrate–methyl blue technic. With this method there is found in the colloid a crystalline substance which differs from that demonstrated with the aid of the two variants of fixation described in the foregoing paragraphs. The amount of this crystalline substance varies not only in the individual thyroid glands examined but even in the individual blocks of the same gland. In one case the crystalline matter is scarce, being found only here and there, in the form of minute needles. In another case it is abundant and appears separated in the form of large needles, rosettes and sheaflike bundles (fig. 3 *E*). This crystalline substance is found mostly in the acini filled with homogeneous and properly stained colloid. Whenever acini are empty or are filled with rarefied and particulated colloid, no crystalline matter is found (fig. 3 *E*). In other words, the acini that are in the phase of active reabsorption or depletion of colloid do not contain crystalline matter. In some instances, however, acini may be filled with homogeneous and properly stained colloid but contain no crystals. The crystals are readily soluble in sodium, ammonium and potassium hydroxide, but they are insoluble in sodium, ammonium and potassium carbonate. They are insoluble in all organic solvents, such as acetone, ether, pyridine, aniline, chloroform and all alcohols. It is apparent that the acetic acid and the iodine employed in this method are responsible for the insolubility of the crystals in alcohol. When heated at the rate of a 10 C increase per minute, their melting point is in the neighborhood of 250 C. Both the specific morphologic aspects and the character of the crystals indicate that they are to be considered as a crystalline thyroxin in the keto form. Crystals of this kind have never been

EXPLANATION OF FIGURE 3

A, section demonstrating peripheral vacuoles containing a crystalline proteolytic enzyme as it appears with the first variant of fixation. × 250. *B*, section demonstrating an apical crystalline proteolytic enzyme as it appears with the first variant of fixation. × 250. *C*, section demonstrating an intravacuolar proteolytic enzyme as it appears with the second variant of fixation. × 250. *D*, section demonstrating a proteolytic enzyme as it appears in the central solitary vacuole with the second variant of fixation. × 250. *E*, section demonstrating intrafollicular crystalline thyroxin in the keto form and as it appears with the third variant of fixation. Note the absence of crystals in the empty follicles, which show remnants of particulated colloid. × 219.

found in organs other than the thyroid gland and they have not been observed in the extrafollicular structures of the thyroid gland. Sometimes, however, they have been seen extrafollicularly in the soft adenomatous gland in which both the vascular and the ground structures are anatomically defective and which shows leakage of the content of acini into surrounding tissues. It is clear from these observations that thyroid glands examined histologically can be classified in regard to their content of thyroxin and that with these methods a number of clinical and experimental problems can be studied with a great deal of certainty and success. It goes without saying that the methods used for pathologic diagnosis and for the study of thyroid problems to date offer no information of similar character.

COMMENT

First of all a few words are to be said in regard to the rationale of the three new variants of fixation used in these studies. Organic dyes, besides having been found of value in histology, have been used successfully in connection with various other problems. Azin and azonium bases, for instance, have been used for the precipitation of bacteriophage and bacterial proteolytic enzymes (Walker [3]). Hershberg and Forbes [4] employed rhodamine B for the precipitation of insulin. Lash and Schönbrunner [5] studied the problem of protecting insulin against the action of digestive ferments by means of various dyes, and they found that certain organic acid dyes can protect insulin against the action of pepsin. Robertson [6] in 1905 was the first to observe that if a drop of saturated solution of safranin is added to a solution of trypsin, a precipitate forms which flocculates in the course of a few hours and collects at the bottom of the tube. This observation was reinvestigated by Holzberg,[7] who found that the precipitate formed has a strong proteolytic action. Investigating chemical aspects of this problem, Marston [8] found that the azin and azonium bases have the power of completely removing the proteolytic enzymes from solutions. The compound formed is stable, and although hardly soluble in ordinary solvents, it is readily soluble in the presence of peptone and protein digestion products. In his opinion this in all probability is due to the combining of the dissociated enzyme ion with the protein digestion products, liberating the azin, which reverts to the orthoquinoid structure, the whole compound thus being decomposed and carried into solution. Marston expressed the belief that the union of the enzyme with azin dyes is a chemical reaction. These briefly outlined observations served to encourage an application of organic dyes to histologic investigations of a similar character. With a variety of dyes being tried, it was found that not one of them could be applied to tissues in aqueous or alcoholic solutions. It was found, however, that incorporation of safranin or toluidine blue into the fixator consisting of dilute solution of formaldehyde and acetone gave, together with fixation, rapid penetration of the dye. An additional treatment with a mixture of solutions of acetic acid and zinc sulfate has been found to be helpful in disclosing certain facts of interest. As compared with Helly's fixator the newly devised fixators cause no shrinking of intrafollicular colloid, and peripheral vacuoles are less numerous. Integration of these observations leads to the conclusion that vacuolation of colloid is caused either by inevitably prolonged surgical operation or by some delay in fixation of tissue. This was corrobo-

3. Walker, A. W.: Proc. Soc. Exper. Biol. & Med. **24**:839, 1927; **34**:726, 1936.
4. Hershberg, H., and Forbes, J. C.: Proc. Soc. Exper. Biol. & Med. **42**:95, 1939.
5. Lash, F., and Schönbrunner, E.: Arch. f. exper. Path. u. Pharmakol. **180**:469, 1935.
6. Robertson, T. B.: J. Biol. Chem. **2**:317, 1906-1907.
7. Holzberg, H. L.: J. Biol. Chem. **14**:335, 1912-1913.
8. Marston, H. R.: Biochem. J. **17**:851, 1923.

rated by the observation of unusually large numbers of vacuoles in the tissues which were fixed following timed delays. In contrast with this peripheral vacuoles were found only seldom in the tissues which were received without undue delay or which were subjected to freezing with solid carbon dioxide for ten minutes followed by fixation with any of the new fixators. The demonstration of crystalline substance in the vacuoles has added to the understanding of the mechanism underlying the formation of vacuoles. It is apparent that the vacuolation is caused by enzymatic liquefaction of colloid and that the crystalline substance found in the vacuoles is an enzyme of proteolytic nature. This process develops with great rapidity from the beginning of surgical injury, and the delay in fixation contributes still more to the extent of liquefaction, which in histologic language is equivalent to vacuolation. The sum of results, then, indicates that the peripheral vacuoles are supravital in origin and that the vacuolation of colloid is a result of enzymatic digestion precipitated by surgical injury and by delay in fixation. It seems reasonable to attribute this process to the activation of enzyme caused by surgical injury or by separation of the gland from its controlling mechanism. In certain instances, in spite of surgical injury and delay in fixation, no peripheral vacuoles are found, and this is apparently due to absence of the enzyme in the follicles.

From time to time a follicle is found which shows a solitary central vacuole. The origin of this vacuole is somewhat different. Its occurrence is not influenced by surgical injury and by delay in fixation, and preliminary freezing remains without influence. Sufficient evidence has been obtained to indicate that it is intravital in origin. It represents a process of removal of central inspissated colloid. All phases of this process could be traced in the material fixed with Helly's fluid and stained with the potassium bichromate–eosin–silver nitrate–methyl blue method. The demonstration of the products of enzymatic digestion in the epithelium of the acini serves as a particularly strong argument in support of this concept. Vacuoles of this type are prominent and frequent in aged people; morever, here they appear mostly black. It seems that the difficulty with removal of inspissated colloid is the main histologically demonstrable feature of the senile thyroid gland.

This new concept in regard to the mechanisms underlying the formation of peripheral and central vacuoles invites discussion of an interesting paper by Williams.[9] His careful studies were made with the aid of an ingenious method which permits direct observation of the follicles of the thyroid gland in living mice. He obtained data which indicate that certain vacuoles are not present in living intact mouse gland free from injury but do appear following such experimental procedures as the application of a gentle stream of cold air or the injection of water, Ringer's solution or certain dyes into the colloid. He observed also that similar vacuoles were formed on interference with the blood supply, and that three hours after death such vacuoles disappeared, all cytologic details became obscure and the colloid became more fluid. Furthermore, he stated that although this vacuolation was a common finding, not all follicles reacted in a similar manner and nothing was found to enable him to predict in what follicle vacuoles would or would not form. It was assumed by Williams that vacuolation is an expression of cellular or colloid reaction to chemical or manipulative procedures. He observed also that under normal circumstances vacuoles were found which he considered to be the ones seen in sections deep in colloid. Their origin was not observed. The findings in the human thyroid glands not only corroborate the correctness of the observations made by Williams but offer in addition concrete

9. Williams, R. G.: Anat. Rec. **79**:263, 1941.

evidence sufficient to explain the mechanisms by which the peripheral and the central vacuoles are formed. Furthermore, in the light of these findings both the absence of vacuoles and the variability in their occurrence in individual follicles can be understood very easily.

The work of Lerman and Salter [10] was the first that suggested the possibility that colloid is reabsorbed from the follicles after enzymatic hydrolysis. They suggested the possibility of an enzymatic mechanism which could catalyze either the formation or the destruction of protein according to thermodynamic conditions. De Robertis [11] demonstrated proteolytic activity in the follicular content of the rat's thyroid gland. The demonstration of this proteolytic activity and of its variation supports, in the opinion of that author, the concept that an enzymatic mechanism is involved in the hydrolysis of the colloid protein and in the subsequent reabsorption of the products of hydrolysis.

Considered alone, the finding of a proteolytic enzyme in the follicular content is not sufficient for the proper understanding of the mechanism of colloid reabsorption or release. Besides enzyme, the follicles must contain a basic substance which is the source of the active hormone. An application of the third variant of fixation, followed by staining with potassium bichromate–eosin–silver nitrate–methyl blue, disclosed in the follicular content a crystalline substance with all the characteristics of thyroxin. Kendall and Osterberg,[12] in their work published in 1919, investigated with particular care all forms of crystalline thyroxin discovered by them. The thyroxin that precipitated in the presence of alkali they named the enol form and that which precipitated in acid solution they named the keto form. These two forms differ in regard to stability and solubility. There are reasons to believe that the crystalline substance demonstrated by me histologically is thyroxin in the keto form. While such crystalline matter was abundant in some follicles, only a little of it was found in others, and in some follicles it was not found at all. Only on rare occasions was it found extrafollicularly. In this connection a reference must be made to the interesting work by Carlson, Hektoen and Schulhof,[13] who demonstrated by means of a precipitin test the presence of thyroglobulin in lymph and in venous blood from the dog's thyroid gland.

The presence or the absence of histologically demonstrable thyroxin is generally independent of the morphologic type of the acinous epithelium. As a rule, however, the follicles depleted in colloid and lined with high columnar epithelium contained no demonstrable thyroxin. Furthermore, it has been found that whenever, regardless of the type of epithelium, the follicles are empty or contain granular particulated colloid, no thyroxin is demonstrable. There can be no doubt that follicles of this appearance are in the phase of release of the active hormone. It is therefore possible to classify cases of thyroid disease in regard to the content of thyroxin. Histologic demonstration of thyroxin is of particular significance from the diagnostic point of view, as it enables one to differentiate between an actually toxic gland, a potentially toxic gland and one that is harmless. Returning to the reabsorption and the release of colloid, one finds that the information obtained is helpful in clarifying this controversial subject. There are two schools of thought in regard to the mechanism of the release of colloid. According to one school, the release is intracellular or, in other words, the colloid is reabsorbed by the acinous epithelium, which in turn discharges it into the circulation. The other school, which backs its opinion with more convincing arguments, states that the

10. Lerman, J., and Salter, W. T.: Tr. Am. A. Study Goiter, 1936, p. 143.
11. De Robertis, E.: Anat. Rec. **80**:219, 1941.
12. Kendall, E. C., and Osterberg, A. E.: J. Biol. Chem. **40**:265, 1919.
13. Carlson, A. J.; Hektoen, L., and Schulhof, R.: Am. J. Physiol. **71**:548, 1925.

colloid passes between epithelial cells and in this way reaches the circulation. The sole objective morphologic evidence in support of the intercellular release of colloid was furnished by Zofya Hirschlerowa.[14] Though her observations concerned only the amphibian thyroid gland, she was able, in the course of her studies on the Golgi apparatus, to demonstrate with the aid of the osmic acid method the intercellular canaliculi. In her opinion the colloid is directed into circulation through these canaliculi, which she called *Ausführungskanalchen* (conductive canals). The other arguments in support of the intercellular release of colloid are indirect: First of all, it is difficult to understand how a molecule with such a large molecular weight as thyroglobulin could pass through a cell. To quote McClendon,[15] diffusion of thyroglobulin with a molecular weight of 700,000, through the plasma membrane of a living cell seems incredible. His own studies, based on direct observation of thyroid tissue in the centrifuge-microscope, led him to the conclusion that the colloid (thyroglobulin) can pass between cells, and he expressed the belief that the mechanism of the release of colloid is intercellular.

The facts disclosed in the course of the present studies not only prove that there is an intercellular mechanism of colloid release but show, in addition, that there are two ways by which colloid is removed from the follicle. While normal colloid is released through the intercellular canaliculi, the inspissated colloid is reabsorbed by the cells of the acinous epithelium. The demonstration in the human thyroid gland of intercellular silver-impregnated canaliculi proves that there is an intercellular mechanism of colloid release. The disclosure of a crystalline enzyme in solitary vacuoles, the morphologic evolution of these vacuoles and the unmistakable demonstration of the digestion products of inspissated colloid in the epithelial cells prove that the removal of this type of colloid is intracellular, and it is therefore reabsorption in the true sense of the word. Interference with either of these two mechanisms of removal of colloid can be easily detected and demonstrated histologically. Whenever there is interference with intercellular removal of colloid, the intercellular canaliculi are found distended, and in advanced cases they may be transformed into small basal intercellular cysts with consequent detachment and desquamation of adjacent epithelial cells. The soft and anatomically defective adenomatous gland particularly is apt to display signs of interference with intercellular removal of colloid. The demonstration of canaliculi is most helpful in determining whether the gland is in the phase of colloid release. Interference with the intracellular mechanism of colloid removal is manifested by the presence of an unusually large number of follicles containing the central vacuoles and by the absence in the epithelial cells of the products of digestion of inspissated colloid. Manifestations of interference with this type of colloid removal are particularly prominent in the thyroid gland of the aged person, and they are actually diagnostic of senility of the gland. It must be admitted that Goldzieher[16] was entirely right in pointing out that the problem of excessive storage of colloid and of defective discharge of colloid was not given the consideration it deserved.

There is a great deal of controversy in the literature as to which particular type of acinous epithelium reabsorbs and which secretes colloid. The results of the present investigation show that normal active colloid is released by way of intercellular canaliculi and that unused, old, central colloid is reabsorbed by

14. Hirschlerowa, Z.: Ztschr. f. Zellforsch. u. mikr. Anat. **6**:234, 1927.

15. McClendon, J. F.: Endocrinology **24**:82, 1939.

16. Goldzieher, M. A.: Practical Endocrinology: Symptoms and Treatment, Section on the thyroid gland, New York, D. Appleton-Century Company, 1936, p. 67.

the epithelial cells, which in these stagnating follicles are usually of the cuboidal type. The demonstration of both the colloid-releasing intercellular canaliculi and the apical secretion granules permits one to determine whether a particular gland is in the phase of colloid release or colloid refilling or whether the gland is functionally quiescent. The observations made on thousands of sections indicate that intercellular release and refilling are not synchronous in regard to the same follicle. Whenever canaliculi are numerous and prominent, apical secretion granules are not found. With the exception of flat, endothelioid epithelium, such apical granules are demonstrated in all types of acinous epithelium, including the high columnar epithelium of the Sanderson polsters. At no time are similar granules found in the basal parts of the epithelial cells.

The significance of each objective finding presented in this publication is self evident. Apart from their theoretic value the findings offer practical information in regard to a number of clinical and experimental problems. The demonstration of a proteolytic enzyme with its relation to surgical trauma may indicate that the post-thyroidectomy reaction is due to excessive accumulation of this activated enzyme and to absorption of it into the circulation. An elevation of the temperature is the main feature of the reaction following an injection of the proteolytic enzyme, and in view of the findings presented and correlated with the clinical data it is probable that the post-thyroidectomy febrile reaction is due to the same factor. As this enzyme is precipitable by certain dyes and thus can be made innocuous, an application of this principle might be of considerable value in treating not only disturbances of the thyroid gland but other pathologic conditions in which a proteolytic enzyme is a contributory or causative factor.

The histologic demonstration of crystalline thyroxin is of great value but, speaking clinically, this finding should be interpreted in conjunction with other findings. The reason for this is that observers do not know the real nature of the thyroid hormone. Harrington,[17] the greatest authority on the chemistry of the thyroid gland, said:

> That this active secretion is thyroxine itself seems unlikely for several reasons. The insolubility of thyroxine is so great that it is difficult to imagine it circulating in the blood in the free condition even in minute concentration. No direct effect of thyroxine on any isolated normal organ or tissue can be demonstrated, and the administration of thyroxine, even intravenously, elicits its effect only after a latent period of astonishing length.

It appears, then, that there must be some enzymatic process involved in the production of physiologically potent hormone.

Nothing so far has been said in regard to the findings in the thyroid glands of the animals examined. In general, they were similar to the findings in the human thyroid glands, but as they were obtained from the examination of normal glands they differed in some respects. The intercellular canaliculi were demonstrated only occasionally, and the extent of vacuolation of the colloid was insignificant in comparison with that observed in the human thyroid glands. Both types of crystals, however, were found in the animal glands. Interesting results, which will be published in a separate communication, were observed in regard to the effect of an extract of the anterior lobe of the pituitary gland containing the thyrotropic factor on the apical and intrafollicular enzyme. They showed that on prolonged injection of this factor the enzyme disappears and the gland at the same time assumes its resting character. It appears likely that it is the exhaustion of the gland in respect to the production of this enzyme that makes the gland nonsensitive to further injection of the thyrotropic factor.

17. Harrington, C. R.: Brit. M. J. **2**:1269, 1936.

In concluding this discussion it must be mentioned that one of the newly devised methods permits histologic demonstration of so-called easily split-off iron, which has been studied extensively by Barkan and Schales.[18] The occurrence of this iron is in direct relation to the degree of surgical trauma and, in fact, it can serve as a detective device for differentiating gentle from rough surgical work. As this subject was studied in regard to various other tissues besides thyroid, it will be reported in a separate communication.

SUMMARY

New methods were devised and applied to the study of human and animal thyroid glands. These methods were found valuable in demonstrating (1) colloid-releasing intercellular canaliculi, (2) intraepithelial apical secretion granules, (3) intraepithelial lipoid granules, (4) argyrophilic reticulum, (5) argentaffin cells similar to those of the intestinal tract, (6) intracellular reabsorption of the central inspissated colloid, (7) an intraepithelial, intravacuolar and intrafollicular proteolytic enzyme in crystalline form, (8) intrafollicular crystalline thyroxine in the keto form, (9) an intravascular and extravascular substance of the nature of heparin, (10) mast cells and (11) easily split-off iron.

Each finding listed has been discussed in regard to various histophysiologic and pathologic problems related to the thyroid gland.

18. Barkan, G., and Schales, O.: Ztschr. f. physiol. Chem. **254**:241, 1938.

ACQUIRED BICUSPID AORTIC VALVE WITH OBLITERATION OF THE COMMISSURAL RAPHE

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The congenital and the acquired biscuspid aortic valve [1] and certain variations of the latter [2] have been described in previous publications. In this paper attention is drawn to the acquired bicuspid aortic valve in which there is complete or almost complete obliteration of the commissural raphe of the combined cusp. This is an advanced stage of the acquired bicuspid valve with a retracted, horizontal raphe. The retraction is due to depression of the attachment of the raphe to the aorta, originally situated at the natural level of the commissures, into the sinus of Valsalva. Not only is the commissural attachment lowered but the entire raphe assumes a position deep in the sinus pocket just above the line of attachment of the combined cusp. In some instances the raphe merges with and into the floor of the sinus and is grossly invisible.

REPORT OF CASES

Four cases of this particular deformity were studied. The obliterated commissure, even though not visible grossly in some of the cases, was located by means of microscopic examination of the relationship between the aortic media and the annulus fibrosus. For this purpose, serial sections were made and studied at intervals of 0.5 mm. The blocks were taken from that part of the aorta and sinus wall just above the attachment of the combined cusp. The sections were longitudinal in 3 cases and transverse in 1 case. They were stained with hematoxylin and eosin and with the combined Weigert and Van Gieson methods for elastic and fibrous tissues.

In each instance the heart was examined for gross and microscopic stigmas of rheumatic disease. Microscopic sections were made according to the method of Gross, Antopol and Sachs.[3] Importance was attached to evidence of valvular lesions, especially vascularity, fibrosis and cellular exudate.

CASE 1.—The patient was a 55 year old white woman who gave a history of fever, chills and malaise of one week's duration. There was a systolic murmur over the entire precordium. She died of septicemia and congestive heart failure three days after admission to the hospital. There was no history of rheumatic fever.

The heart weighed 350 Gm. The aortic valve consisted of two cusps, each measuring 3.5 cm. in length (fig. 1). One was a combined left and right coronary cusp whose sinus of Valsalva contained no raphe. The central part of the sinus pocket was covered with friable flat grayish red vegetations. Similar vegetations were present at the base and outer part of the noncoronary cusp.

Both aortic cusps were thickened and moderately rigid. At the base of the cusps were numerous confluent calcific nodules projecting into the sinus of Valsalva. At commissure B [4] there was a slight adhesion. Commissure C showed no change.

Longitudinal microscopic sections through the midportion of the combined cusp revealed complete reversal of the aortic media–annulus fibrosus relation. This took place in a seg-

From the Institute of Pathology, Western Reserve University, and University Hospitals of Cleveland.

1. Koletsky, S.: (*a*) Arch. Int. Med. **67**:129, 1941; (*b*) **67**:157, 1941.

2. Koletsky, S.: Am. J. Path. **19**:395, 1943.

3. Gross, L.; Antopol, W., and Sachs, B.: Arch. Path. **10**:840, 1930.

4. The nomenclature of the aortic valve used is as follows: The aortic cusps are designated according to the situation of the coronary arteries as the left, the right and the noncoronary cusp. The left-right commissure is referred to as commissure A; the right-noncoronary commissure, as commissure B, and the left-noncoronary commissure, as commissure C.

ment of aorta about 4 mm. in width just above the attachment of the cusp. The media was anterior to the annulus to either side of this segment and within it gradually assumed a position behind the annulus. The latter occurred approximately at the center of the combined cusp.

Microscopically, the aortic valve showed chronic inflammation and superimposed acute bacterial endocarditis. The remaining valves revealed no significant gross or microscopic change.

CASE 2.—The patient was a white man 43 years old who died of congestive heart failure. The clinical diagnosis was rheumatic heart disease with aortic stenosis and insufficiency as well as mitral insufficiency. There was a history of rheumatic fever at the age of 18.

The heart weighed 650 Gm. The aortic valve consisted of two cusps, each measuring 4.5 cm. in length (fig. 2). One of these corresponded to a combined left and right coronary cusp. No raphe was found in its sinus of Valsalva, and the site of commissure A was not apparent. In the center of the sinus floor the valvular attachment was bridged by a barely visible, slightly elevated fibrous plaque 4 mm. in width. The distal extremity of this plaque was situated opposite a retraction at the base of the ventricular aspect of the combined cusp.

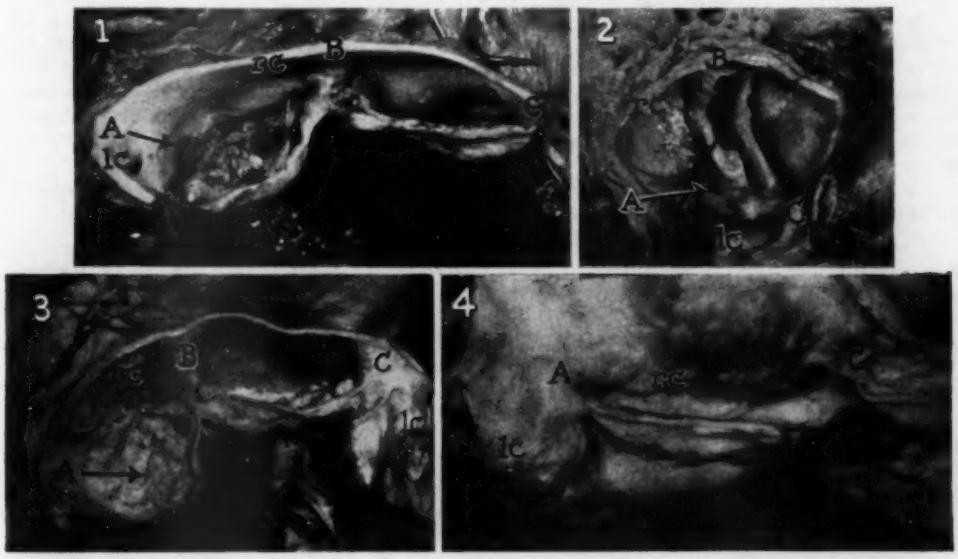

Fig. 1 (case 1).—Acquired bicuspid aortic valve from a white woman 55 years old. The valve is the seat of calcific disease and acute bacterial endocarditis. Commissure A is obliterated. In this and succeeding figures *A, B* and *C* represent the left-right, the right-noncoronary and the left-noncoronary commissures, respectively, while *lc* and *rc* indicate the ostia of the left and right coronary arteries, respectively. An arrow indicates the site of the obliterated commissure of the combined cusp as determined by microscopic study.

Fig. 2 (case 2).—Acquired bicuspid aortic valve from a white man 43 years old. It shows calcific disease with stenosis. Commissure A is not visible.

Fig. 3 (case 3).—Calcific stenosis of the acquired bicuspid aortic valve of a white man 84 years old. Commissure A could not be identified grossly.

Fig. 4 (case 4).—Syphilis and endocarditis lenta of the acquired bicuspid valve of a white man 35 years old. Commissure B was obliterated in the sinus pocket of the combined cusp.

Both aortic cusps were thick, rigid and slightly retracted, and showed extensive calcific deposit. The latter was widely distributed in the substance of the cusps and extended from the base to the line of closure. There was slight adhesion at commissure B. Commissure C showed no change.

Longitudinal microscopic sections of the root of the aorta showed a commissural type of overlap between aortic media and annulus fibrosus opposite the center of the combined cusp. This was situated 10 mm. below the upper commissural level and 5 mm. above the attachment

of the cusp. The annulus was anterior to the media. A short distance to either side of the commissure the junction became first horizontal and then changed to a relation of media anterior to annulus.

The mitral valve was the seat of slight stenosis. Microscopically, rheumatic stigmas were present in all four valves and in the left atrium.

CASE 3.—The patient was a white man aged 84 who died of cerebral hemorrhage. There was a clinical diagnosis of rheumatic heart disease with aortic stenosis and insufficiency. No history of rheumatic fever was obtained.

The heart weighed 400 Gm. The aortic valve was bicuspid (fig. 3). The larger cusp was a combined left and right coronary cusp measuring 4.5 cm. in length. The smaller or noncoronary cusp measured 4 cm. in length. No definite raphe could be identified in the sinus of Valsalva of the combined cusp. At the valvular attachment near the middle of the sinus pocket there was a tiny horizontal ridge, slightly raised and barely perceptible. This was situated 2 cm. from commissure B and 2.5 cm. from commissure C, opposite a retraction toward the aorta of the basal portion of the combined cusp.

The aortic cusps were thickened and slightly retracted, and showed considerable calcific deposit. The latter was found chiefly in the sinus pockets at the base of the cusps, but also involved the line of closure. Commissures B and C revealed no adhesion.

A complete reversal of the aortic media–annulus fibrosus relation was demonstrated by longitudinal serial sections of the combined cusp. In the root of the aorta, a short distance above the attachment of the left central portion of the cusp, the terminal aortic wedge was situated posterior to the annulus fibrosus. A few millimeters to each side of this arrangement the media occupied a position anterior to the annulus and descended almost to the valvular attachment. The commissure was located about 2 mm. to the left of the tiny ridge described in the gross specimen.

Gross rheumatic disease was limited to the aortic valve. Microscopically, the aortic, mitral and pulmonary valves and also the left atrium showed rheumatic stigmas.

CASE 4.—The patient was a 35 year old white man with a history of cardiac failure of three months' duration. The clinical diagnosis was rheumatic heart disease with aortic stenosis and insufficiency. Death was due to congestive heart failure. There was no history of rheumatic fever.

The heart weighed 550 Gm. There was syphilitic disease of the aorta and the aortic valve. The latter consisted of two cusps measuring 4 and 2 cm. in length, respectively (fig. 4). The larger represented a combined right coronary and noncoronary cusp. No raphe was found in the sinus of Valsalva. Commissure B could not be identified. However, two shallow grooves, several millimeters apart, were present in the central part of the floor of the sinus pocket. These passed obliquely across the valvular attachment, extending from the aorta to the base of the combined cusp, and diverged in their distal portions.

The aortic valve was the seat of endocarditis lenta. Both cusps were thickened, retracted and slightly rigid. No calcific deposit was present. Commissures A and C each showed a separation of 3 mm.

Serial transverse microscopic sections were made of the aortic wall of the sinus pocket of the combined cusp. These extended from the normal commissural level to the attachment of the cusp. The junction of aortic media and annulus fibrosus at commissure B occurred about 5 mm. above the valvular attachment at the center of the cusp. The elastica was situated behind the annulus and in contact with the pericardium.

The mitral valve showed chronic nondeforming rheumatic valvulitis. Microscopic stigmas of rheumatic disease were limited to the aortic and mitral valves.

SUMMARY OF CASES

Three of the patients were men and 1 was a woman. The ages ranged from 35 to 84 years. In 3 cases there was a clinical diagnosis of aortic stenosis and insufficiency. One patient gave a history of rheumatic fever.

In all cases the aortic valve showed pathologic change. Calcific disease of the valve was present in 3 cases, in 1 of which the valve was also the seat of acute bacterial endocarditis. In 1 case there was combined rheumatic and syphilitic disease together with endocarditis lenta.

The combined cusp of the aortic valve was formed by fusion of the left and right coronary cusps in 3 cases and by fusion of the right coronary and posterior cusps

in 1 instance. In 2 cases the combined cusp was equal in size to the second cusp, and in 2 it was larger.

The obliterated commissure was situated in the root of the aorta at a point opposite the center of the combined cusp in 3 cases and opposite the left central portion in 1 case. In every instance this site was well below the position of the other commissures, i. e., in the lower third of the aortic wall of the sinus of Valsalva or just above the valvular attachment.

The gross and microscopic stigmas of rheumatic disease are shown in the accompanying table. Such stigmas were present in all 4 cases. In 1 case they were confined to the aortic valve.

Summary of Four Cases of Acquired Bicuspid Aortic Valve with Obliterated Commissural Raphe

Case	Patient	Clinical Diagnosis (Aortic Valve)	Location of Obliterated Raphe	Disease of Aortic Valve	Stigmas of Rheumatic Disease	
					Gross	Microscopic
1	55 yr. old white woman	No diagnosis made	Commissure A	Calcific disease; acute bacterial endocarditis	Aortic valve	Aortic valve
2	43 yr. old white man	Aortic stenosis and insufficiency	Commissure A	Calcific disease	Aortic and mitral valves	All four valves, left atrium
3	84 yr. old white man	Aortic stenosis and insufficiency	Commissure A	Calcific disease	Aortic valve	Aortic, mitral and pulmonary valves, left atrium
4	35 yr. old white man	Aortic stenosis and insufficiency	Commissure B	Rheumatic and syphilitic disease, endocarditis lenta	Aortic and mitral valves	Aortic and mitral valves

COMMENT

Although the commissural raphe in this type of bicuspid aortic valve is obliterated, certain vestiges may indicate its location on gross inspection. They usually occur at or near the middle of the sinus pocket of the combined cusp, especially near that portion of the base of the cusp which is retracted toward the aorta. They include irregular elevation of the floor of the sinus, oblique grooving of the terminal portion of the aorta, and a slight, barely perceptible ridge which passes across the valvular attachment.

However, such vestiges are not conclusive. Similar changes may result from inflammation or calcific deposition, especially the formation of a ridge.[2] Proof that the raphe existed at a given site is obtained by demonstrating the presence of a commissure. This is shown microscopically by the relation of the aortic media to the annulus fibrosus of the aortic valve.

Normally the aorta terminates as a wedge-shaped projection which meets a similar upward projection of the annulus fibrosus just above the attachment of the aortic cusps.[5] The junction occurs on a roughly oblique plane. In the sinus pocket corresponding to the midportion of each cusp, the aortic wedge lies anterior to the annulus and distal to the pericardial mantle. Laterally, as the commissure is approached, this arrangement undergoes complete reversal. Near the commissure the plane of junction is essentially horizontal, while at the commissure proper the terminal wedge of aorta occupies a position posterior to the annulus and proximal to the pericardium. Gross [6] referred to this change of position as normal inversion.

5. Lewis, T., and Grant, R. T.: Heart **10**:21, 1923.
6. Gross, L.: Arch. Path. **23**:350, 1937.

Infrequently, a more or less horizontal junction between media and annulus fibrosus persists at the commissure.

The relation between the aorta and the annulus fibrosus at the commissure is not altered when a raphe forms because of adhesion of cusps. The same holds true for the commissural raphe of the acquired bicuspid aortic valve, both the usual form [1b] and the retracted, horizontal type.[2] At the proximal extremity of the raphe the aortic media terminates behind the annulus. This arrangement is maintained even when the terminal elastica and the annulus are distorted by scarring and calcific deposit. Gross [6] attributed lack of normal inversion of the aorta in 2 of 18 raphes from bicuspid aortic valves to inflammation, but a congenital lesion was not excluded in either one. Normal inversion of the terminal aortic wedge is retained in the acquired bicuspid aortic valve. From its posterior position relative to the annulus fibrosus at the commissural raphe, the wedge reverts to an anterior position within several millimeters to either side of the raphe.

An obliterated raphe can also be identified by means of the relationship between aortic media and annulus fibrosus. This requires microscopic study of the segment of aorta containing the former site of attachment of the raphe, i. e., usually the segment above the attachment of the midportion of the combined cusp. Serial longitudinal sections of this region show reversal of position of the terminal portion of the aorta with respect to the annulus. The commissure is situated where the aortic wedge lies posterior to the annulus. A single longitudinal section, if obtained in the right place, can disclose this site. Probably a single transverse section of the aorta made immediately above, or a short distance above, the valvular attachment of the combined cusp would demonstrate the commissure more readily. In a normal cusp it would show a continuous elastic layer anterior to the annulus fibrosus, whereas at a commissural site the elastica would be interrupted anteriorly by the annulus.

The anterior overlapping of the annulus fibrosus by the terminal aortic wedge just above the attachment of the midportion of each normal aortic cusp is constant. In valves the seat of inflammation distortion of both the elastica and the annulus may result from scarring, intimal fibrosis of the terminal portion of the aorta and calcific deposit. Such changes produce irregularity at the site of junction, but the portion of annulus which projects up behind the aorta can still be identified.

Prior to obliteration the raphe in each of the cases in this study was attached to the aorta at a point well below the normal position of the commissure. This resulted from downward displacement of the proximal extremity from its original location at the commissure. Such a raphe assumes a horizontal position deep in the sinus of Valsalva in contrast to that observed with the usual type of acquired bicuspid aortic valve, which is directed obliquely in the sinus. Although lowering of the commissural attachment occurred at commissure A in 3 of the present 4 cases, my experience is that such lowering is more frequent at commissure B for both the rheumatic raphe and that of the acquired bicuspid aortic valve.[2] No instance of obliteration of the oblique form of raphe has been encountered.

To be distinguished from the acquired bicuspid aortic valve with obliterated commissural raphe is the simple congenital bicuspid aortic valve, i. e., the valve with two normal cusps. Gross vestiges of the obliterated raphe may be of aid in this respect, except in cases of congenital bicuspid valve with extensive calcific deposit in the sinuses of Valsalva. Differentiation can be made microscopically, since the arrangement of the terminal portion of the aorta and the annulus fibrosus is the same for the congenital lesion as for the normal aortic valve.

The acquired bicuspid aortic valve is the result of an inflammatory process usually, if not always, of rheumatic origin.[7] Stigmas of rheumatic disease occur in the valve and are usually found elsewhere in the heart. In 1 of the 4 cases in this study the stigmas were apparently limited to the aortic valve, while in 3 cases additional lesions were present, especially in other valves (table).

The bicuspid lesion is frequently accompanied by calcific disease or bacterial endocarditis. The former occurred in 3 of the present cases and the latter in 2 cases. Deposition of calcium constitutes an integral part of the rheumatic lesion. Bacterial disease is presumably superimposed on the bicuspid aortic valve because of the rheumatic orgin.[8]

SUMMARY AND CONCLUSIONS

The acquired bicuspid aortic valve with obliterated commissural raphe probably represents a further stage of the acquired bicuspid aortic valve with retracted, horizontal raphe. In the 4 cases described the obliterated commissure was identified by means of the aortic media–annulus fibrosus relationship.

The lesions are usually of rheumatic origin. Conclusive stigmas of rheumatic disease were found in the heart in 3 cases, while in 1 case there were probable stigmas, limited to the aortic valve.

In 3 cases the aortic valve showed calcific disease with stenosis. Bacterial endocarditis was present in 2 cases.

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7. Koletsky.[2] Gross.[6]

8. Koletsky, S.: Bicuspid Aortic Valves and Bacterial Endocarditis, Am. Heart J. **26**:343, 1943.

ABDOMINAL VISCERAL LESIONS ASSOCIATED WITH PRIMARY DISEASE OF THE LIVER

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During the past few years we have observed the occurrence of pancreatic fat necrosis and extensive inflammatory lesions of the intestinal tract in instances of primary disease of the liver. The severity of these lesions appeared to be more than fortuitous. The pathologic alteration of the intestinal tract was particularly striking and unlike any that we had previously encountered.

The occurrence of lesions of the abdominal viscera in primary disease of the liver is well known. Interstitial inflammation of the pancreas is almost universal in all forms of cirrhosis and is frequently present in acute or subacute yellow atrophy of the liver. Likewise, circulatory alterations of the bowel as a result of thrombosis of the portal or the mesenteric vein are occasionally seen. However, phlegmonous inflammation of the intestinal tract such as we have observed does not appear to have been described. Nor has any mention been made of the presence of pancreatic fat necrosis in association with hepatic disease. In order to determine whether these lesions were merely incidental or were significantly associated with hepatic disease we reviewed the gross and microscopic observations in a number of selected cases.

MATERIAL

Eighty-eight cases of primary disease of the liver in which adequate material was available were studied. These included 49 cases of Laennec's cirrhosis, 26 of acute and subacute yellow atrophy of the liver, 12 of toxic (coarse nodular) cirrhosis and 1 of cholangiolitic cirrhosis.

OBSERVATIONS

The occurrence of pancreatic fat necrosis is summarized in the table. A case was classified as showing extensive necrosis when there was grossly massive involvement of the organ. The severity was identical with that seen in so-called hemorrhagic pancreatitis. The term "moderate pancreatic fat necrosis" was used to indicate those instances in which there were smaller or larger circumscribed areas of involvement. The term "minimal" was applied to indicate small foci of pancreatic

Incidence of Pancreatic Fat Necrosis and Phlegmonous Enteritis in Primary Disease of the Liver

		Necrosis of the Pancreas			Inflammation of the Intestinal Wall		
Disease	Cases	Extensive	Moderate	Minimal	Severe	Moderate	Comment
Laennec's cirrhosis.....	49	2	1	10	6	6	No intestinal sections available in 16 cases
Yellow atrophy (acute and subacute)	26	3	2	2	2	0	No intestinal sections available in 5 cases
Toxic cirrhosis (coarse nodular)	12	0	2	0	1	2	
Cholangiolitic cirrhosis	1	0	0	0	1	0	

From the Laboratories of the Mt. Sinai Hospital.

fat necrosis, often observed only microscopically. The gross and microscopic appearance of pancreatic fat necrosis is too well known to require further description.

An analysis of the frequency of occurrence of pancreatic fat necrosis in a control group of 100 postmortem cases in which the age group would correspond to that of our series and in which the cases were unselected except for exclusion of those with primary pancreatic disease revealed but 1 instance of moderate pancreatic fat necrosis and a number with minimal necrosis.

The lesion of the intestinal tract when first seen was not fully appreciated. We were struck by a marked bogginess of the small and large intestine. Because of the presence of ascites, this peculiar appearance was attributed to edema in some of the earlier cases. The degree of edema was sufficiently profound to warrant a special notation in the gross anatomic description. On microscopic examination,

Low power view of small intestine showing diffuse phlegmonous inflammation with pronounced edema.

however, there was seen a diffuse, phlegmonous inflammation of the intestinal wall characterized by an infiltration of all the layers by polymorphonuclear leukocytes (fig.). This appearance was more or less uniform throughout the small and large intestine. Subsequent to these first experiences, when the same peculiar boggy appearance of the intestinal tract was again encountered, the characteristic microscopic appearance was predicated. The frequency of the intestinal lesion is stated in the table. It was noted ten times in a severe and eight times in a moderate degree. We have not encountered this lesion during the past ten years except in association with primary disease of the liver as tabulated.

The pancreatic fat necrosis and the inflammatory lesion of the intestine were found concurrent in 5 cases.

COMMENT

In an attempt to determine what possible factors might underly the changes in the pancreas and the intestine in primary disease of the liver, certain general and local alterations must be considered. Jaundice was present at the time of death in all but 3 cases; these were cases of Laennec's cirrhosis. Hence jaundice per se cannot be considered as a causative factor. Similarly, it is evident that degeneration of the liver is not a deciding factor, since it also was common to many cases of primary disease of the liver unattended by pancreatic and intestinal lesions.

When we consider the pancreatic and intestinal lesions separately, there are certain local conditions which present themselves as possible causative factors. Nevertheless, in none of these cases were there found the usual factors which have been cited to explain the occurrence of pancreatic fat necrosis, such as obstructions due to inflammation, stone of the pancreatic or the common duct, or tumor.

The greater frequency of pancreatic fat necrosis in primary disease of the liver as compared with a miscellaneous group of conditions is readily apparent. This association lends credence to the view that the lesion in the pancreas might have some common basis with that in the liver. The extensive pancreatic lesions were found most frequently in that group of cases, viz. cases of yellow atrophy and toxic cirrhosis, in which primary degeneration is the most significant alteration of the liver. The possibility suggests itself that the same factors that play a role in producing hepatic degeneration may simultaneously affect the pancreas.

Attention has recently been directed by Rich and Duff [1] to the occurrence of squamous metaplasia as a possible basis for fat necrosis of the pancreas. This was found only twice and then in a relatively insignificant degree in our series. Furthermore, we have seen squamous cell metaplasia of the pancreatic ducts as a purely incidental observation without any associated pathologic alterations.

There were no significant local vascular lesions. Thrombosis of the portal or the splenic vein occurred in 3 cases. However, with a single exception, only 1 instance of pancreatic fat necrosis with phlegmonous inflammation of the bowels showed thrombosis of the splenic vein; in the remainder the splenic and portal veins were normal. In the excepted instance the thrombosis was of recent origin. It is likely that the thrombosis followed the pancreatic lesion. A review of our cases of primary pancreatic fat necrosis shows the rather frequent associated occurrence of thrombosis of the splenic vein as a complication. Similarly we have observed a number of instances of primary thrombosis of the splenic vein without any secondary pancreatic lesions. One of our cases of acute yellow atrophy associated with massive pancreatic fat necrosis showed a large subphrenic abscess at a recent site of splenectomy. It cannot be fully decided whether the pancreatic lesion in this case was subsequent to the infection or to the operative procedure. In 1 other instance minimal fat necrosis was associated with tumor thrombosis of the splenic vein secondary to primary carcinoma of the stomach. Thus, it is evident that in only 3 of our 19 cases was pancreatic fat necrosis associated with any local lesion which might be construed as causal.

Pronounced ascites was present in some of the cases of hepatic cirrhosis; minimal to moderate amounts of peritoneal fluid were seen in cases of yellow atrophy. However, there was no correlation between the degree of ascites and the phlegmonous lesion in the intestinal tract. Bacterial strains of the intestinal wall revealed gram-negative bacilli in 1 instance and gram-positive cocci in several. The significance of this for the occurrence of infected ascites is evident. The

1. Rich, A. R., and Duff, G. L.: Bull. Johns Hopkins Hosp. **58**:212, 1936.

mechanism is comparable to that seen in migration peritonitis. These bacteria may infect the ascitic fluid by migrating through the intestinal wall. No doubt, in some instances the infection of ascitic fluid in cirrhosis, which has been attributed to contamination following paracentesis, might well have had its origin in such an intestinal lesion. We were most careful to evaluate the relation of incidental peritonitis to the phlegmonous lesion of the bowel. Several instances of peritoneal inflammation of varying degrees were seen without any associated phlegmonous enteritis.

There was no correlation between the degree of interstitial pancreatic or hepatic inflammation and the occurrence of pancreatic fat necrosis or phlegmonous inflammation of the intestinal tract.

Inasmuch as these studies were principally pathologic in character, significant clinical observations were not made. Many of these patients were quite ill, but no specific symptoms were observed. Because of the ascites and abdominal distention usually noted in hepatic disease, it was not possible to determine what part the intestinal lesions played in the production of these symptoms. Mention is made by Anschütz [2] of ileus occurring in 6 cases of cirrhosis of the liver. Two of the patients came to postmortem examination, but in neither was any bowel lesion described. It is likely, however, that in subsequent clinical observations it might be possible to attribute intractable ileus to such intestinal inflammation. Similarly sudden collapse of a patient with yellow atrophy might speak for coexistence of extensive fat necrosis of the pancreas. Further clinical studies are necessary before the role of these complications can be established in the complicated course of severe hepatic disease.

SUMMARY

Fat necrosis of the pancreas and a phlegmonous lesion of the intestine have been observed in instances of primary diseases of the liver. These lesions appeared to bear no relation to the degree of hepatic inflammation. They were more frequent, however, in instances in which severe hepatic degeneration was noted. They may have clinical significance.

2. Anschütz, W.: Acta chir. Scandinav. **71**:32, 1932.

EXPERIMENTAL ATHEROSCLEROSIS

V. EFFECT OF TESTOSTERONE PROPIONATE AND ESTRADIOL DIPROPIONATE ON THE CHOLESTEROL CONTENT OF THE BLOOD AND THE AORTA IN CASTRATE FEMALE RABBITS

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In a recent communication from this laboratory it was shown that testosterone propionate and estradiol dipropionate administered to female rabbits fed cholesterol inhibited the development of hypercholesteremia and prevented deposition of cholesterol in the aorta; no such protective action was noted in male rabbits.[1] Since it was demonstrated that an androgen and an estrogen were equally effective in preventing deposition of cholesterol in the female animal, the assumption was made that both these steroids produced a common metabolic effect through some obscure mechanism which takes place in the female but not in the male animal. It was an obvious and logical step to determine whether the female gonads were necessary for the exhibition of this protective property by the steroid hormones.

MATERIAL AND METHODS

Observations on 26 female rabbits (3 to 5 months old) which survived castration and one hundred days of the experimental period form the basis for the present report. The animals were kept in individual cages and were fed an adequate amount of a commercial rabbit chow[2] daily. They were castrated[3] under ether anesthesia from twenty-one to twenty-eight days before the experiment was begun. Testosterone propionate and estradiol dipropionate[4] were administered intramuscularly in dilutions so adjusted that each dose was dissolved in 1 cc. of sesame oil. The injections were given daily during the first week and three times weekly during the subsequent thirteen weeks of the experiment. Cholesterol when fed was mixed intimately with the food.

The animals were divided into groups as follows:

A—3 rabbits: 1 cc. of sesame oil three times weekly.

B—4 rabbits: 10 mg. of testosterone propionate three times weekly.

C—3 rabbits: 0.2 mg. of estradiol dipropionate three times weekly.

D—6 rabbits: 1 cc. of sesame oil and 1 Gm. of cholesterol three times weekly.

E—4 rabbits: 10 mg. of testosterone propionate and 1 Gm. of cholesterol three times weekly.

F—6 rabbits: 0.2 mg. of estradiol dipropionate and 1 Gm. of cholesterol three times weekly.

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1. Ludden, J. B.; Bruger, M., and Wright, I. S.: Arch. Path. **33**:58, 1942.

2. This chow is prepared by the Ralston Purina Company, St. Louis, and is said to contain a mixture of grains and alfalfa hay supplemented by vitamins and minerals.

3. The ovaries, fallopian tubes and one-half of the uterus were removed. The operative mortality rate was 21 per cent.

4. The testosterone propionate (Perandren) and the estradiol dipropionate (Diovocylin) were supplied by the Ciba Pharmaceutical Products, Inc., Summit, N. J.

The whole blood cholesterol was determined every eight to fourteen days by Bloor's method as modified by Sackett.[5] The procedure used for killing the rabbits, the technic for removal of the aorta and the method employed for determining the cholesterol content of the aorta have been described elsewhere.[6]

RESULTS AND COMMENT

Table 1 shows that castration per se did not alter the cholesterol content of the blood or that of the aorta in female rabbits (group A). Moreover, 450 mg. of

TABLE 1.—*The Cholesterol Content of the Whole Blood and of the Aorta in Castrate Female Rabbits Receiving Sesame Oil (Group A), Testosterone Propionate (Group B) and Estradiol Dipropionate (Group C)*

		Whole Blood Cholesterol, Mg. per 100 Cc.										Aorta Cholesterol, Mg. per 100 Gm.
		Days										
Group	Rabbit	0	8	22	36	50	64	78	92	100	Mean	Dry Weight
A. Sesame oil.....	374	95	99	108	81	...	107	88	123	108	101	472
	375	175	89	121	86	...	114	144	138	127	124	386
	376	149	109	85	110	...	124	152	123	124	122	370
	Average	140	99	105	92	...	115	128	128	120	116	409
B. Testosterone propionate	362	116	108	103	85	...	102	105	95	86	100	520
	363	83	95	80	93	...	66	89	90	74	84	382
	364	94	144	90	92	...	114	141	104	88	108	383
	365	119	89	124	123	...	104	86	119	112	110	367
	Average	103	109	99	98	...	97	105	102	90	101	413
C. Estradiol dipropionate	369	93	94	112	87	...	107	87	144	100	111	340
	377	94	82	...	97	109	107	108	96	100	99	425
	180	83	142	101	107	105	107	128	160	156	121	...
	Average	90	106	107	97	107	107	108	133	139	110	383

TABLE 2.—*The Cholesterol Content of the Whole Blood and of the Aorta in Castrate Female Rabbits Fed Cholesterol and Receiving Sesame Oil (Group D), Testosterone Propionate (Group E) and Estradiol Dipropionate (Group F)*

		Whole Blood Cholesterol, Mg. per 100 Cc.										Aorta Cholesterol, Mg. per 100 Gm.
		Days										
Group	Rabbit	0	8	22	36	50	64	78	92	100	Mean	Dry Weight
D. Sesame oil and cholesterol	83	115	104	239	300	412	310	446	636	682	360	845
	4	123	364	852	1,106	1,072	1,163	1,000	915	605	800	4,525
	22	156	306	493	507	514	395	559	605	765	478	1,808
	6	91	171	338	475	586	417	562	682	852	464	590
	379	90	119	...	205	375	615	781	962	938	522	6,754
	339	86	186	...	221	329	298	441	412	544	315	630
	Average	110	209	481	483	548	533	632	702	731	490	2,525
E. Testosterone propionate and cholesterol	353	98	158	181	...	226	357	379	308	278	248	556
	79	129	223	408	815	1,000	1,042	1,064	893	1,042	735	1,738
	358	132	197	332	264	379	272	451	387	514	325	463
	954	140	357	893	781	879	794	714	500	636	633	1,106
	Average	125	234	454	620	621	616	652	522	618	485	966
F. Estradiol dipropionate and cholesterol	378	130	186	313	514	...	670	988	670	765	530	2,342
	355	102	204	289	391	463	400	469	421	568	367	698
	356	96	162	421	387	408	408	400	412	487	353	1,175
	357	131	303	670	962	962	820	938	1,014	1,250	783	4,525
	94	114	229	658	682	794	781	781	815	1,111	663	3,189
	181	130	177	184	291	417	708	915	708	596	458	...
	Average	117	210	423	538	609	631	749	673	796	526	2,386

testosterone propionate and 9 mg. of estradiol dipropionate administered in divided doses over a period of one hundred days likewise failed to influence the concentration of this sterol in the blood and the aorta (groups B and C). Gonadectomy, therefore, does not alter the response of female rabbits to those two steroid hormones

5. Sackett, G. E.: J. Biol. Chem. **64**:203, 1925.
6. Bruger, M., and Fitz, F.: Arch. Path. **25**:637, 1938.

since comparable observations were made by Ludden, Bruger and Wright [7] in noncastrate female rabbits.

Table 2 shows that young castrate female rabbits fed cholesterol testosterone propionate and estradiol dipropionate do not inhibit the rise in blood cholesterol nor do they prevent the production of atherosclerosis of the aorta (groups E and F). It is questionable whether testosterone is mildly protective in regard to the quantity of cholesterol deposited in the aorta (compare groups D and E); this steroid does not abrogate the hypercholesteremia in cholesterol-fed female castrates. Since it was previously shown that the steroid hormones, testosterone and estradiol, inhibit the rise in blood cholesterol and prevent the deposition of this sterol in the aorta in noncastrate female rabbits fed cholesterol,[1] it follows that castration tends to nullify this response. The presence of the female gonads, therefore, appears to be a necessary prerequisite for these steroid hormones to exert their protective action. In a measure, our observations were not unlike those reported by Turner and Khayat,[8] which demonstrated that whereas potassium iodide prevents the usual hypercholesteremia and atherosclerosis of the aorta in normal cholesterol-fed rabbits, thyroidectomy abolishes this protective property.

SUMMARY

Castration per se does not alter the cholesterol content of the blood or that of the aorta in young female rabbits.

Testosterone propionate and estradiol dipropionate administered at frequent intervals over a period of one hundred days fail to influence the cholesterol content of the blood or that of the aorta in female castrates.

Feeding cholesterol produces hypercholesteremia and increased deposition of cholesterol in the aorta regardless of the presence or the absence of the female gonads.

Testosterone propionate and estradiol dipropionate inhibit the hypercholesteremia and prevent the excess deposition of cholesterol in the aorta of the female rabbit fed cholesterol but when the gonads are removed this protective action is abolished.

7. Ludden, J. B.; Bruger, M., and Wright, I. S.: Endocrinology **28**:999, 1941.
8. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.

NEUROPATHOLOGY OF MALNUTRITION ASSOCIATED WITH PROLONGED ALCOHOLISM

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The histology of adults dying from malnutrition can stand cautious augmentation. The complexity of the clinical syndromes of nutritional deficiency probably is more responsible than all other factors for the varying neurologic changes that have been recorded for this condition.

From among cases of death of an adult from malnutrition 2 have been selected for thorough histologic analysis. They were relatively uncomplicated cases of avitaminosis except for the association of prolonged alcoholism. Both patients were women who obviously suffered from deficiency of a number of food factors, as is most certainly true of any one dying of malnutrition. In the first case the clinical diagnosis was polyneuropathy and Korsakoff's psychosis, and in the second, pellagra.

CASE 1

A 40 year old white housewife entered the Cincinnati General Hospital on March 4, 1937. She was said to have been in good health until January 1937, when she contracted an infection of the upper respiratory tract. Then she lost her appetite and steadily lost weight. She became increasingly weak and hoarse and deteriorated mentally. She had been up and about until two weeks before admission to the hospital, since which time she had been bedfast and had ingested little or no food. She had been unsteady on her feet for three weeks before going to bed. Prior to her illness her diet as described by an alcoholic husband was considered to be adequate. It was said to contain a wide variety of meats and green vegetables and was said to have been ingested in large quantities.

The patient had been a heavy consumer of alcoholic beverages for as long as her husband knew anything about her. For at least twenty years she had drunk a pint (473 cc.) of whisky a day. She increased her consumption to more than one pint a day with the repeal of prohibition (1932). She was said to have never been drunk, that "she held liquor well." During her last illness she discontinued taking whisky, substituting wine, and refused the latter for about one week before her entrance to the hospital. She had never had syphilis and had never miscarried. She had borne two children, who died in infancy.

On admission she was markedly emaciated. She had lost 15 to 20 pounds (6.5 to 9 Kg.) within six weeks, her best weight having been 107 pounds (48.5 Kg.) six weeks before entry. The abnormal findings besides emaciation were a fever of 1 degree (F.), a pulse rate of 140 and dehydration. The blood pressure was 122 systolic and 86 diastolic. She was uncooperative and occasionally became deliriously noisy or profane. She spoke infrequently but moved her lips as though in conversation. She appeared to be having visual and auditory hallucinations. There was bilateral ptosis. The pupils were 7 mm. in diameter and reacted through a small range to flashlight. The extremities executed continuous rapid tremulous purposeless movements. It was thought that resistance to passive movement was increased in the extremities, though this point was difficult to test with accuracy. There was complete foot and wrist drop on both sides. It was not possible to make further motor or sensory examinations. She did not respond to pinprick anywhere. The tips of the fingers and toes were bright red. The tongue was inflamed and was smooth at the tip. The tendon reflexes in the arms and the left knee jerk were hyperactive. The right knee jerk and the

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ankle jerks were not obtained. The plantar responses were extensor on early examinations; later the plantar responses were flexor and were accompanied by some reflex withdrawal.

The temperature, the pulse rate and the respiratory rate rose gradually during the course in the hospital. She continued to be irrational, restless and tremulous, and she was noisy on occasions. Despite vigorous supportive therapy, she died on March 8, 1937, the fifth day in the hospital.

Apart from two red blood cell counts of 4,000,000 and two hemoglobin determinations of 8.5 Gm. the laboratory findings were of no significance. The Kahn test of the blood was negative.

The clinical diagnosis was malnutrition and vitamin deficiency, chronic alcoholism, polyneuropathy and Korsakoff psychosis.

Abnormal tissue changes besides those in the nervous system included marked fatty infiltration of the liver and erosion of the mucosa of the ileum. The mucosa of the ileum contained many denuded areas, and the lining of the remainder of the gastroenteric tract was slightly congested but otherwise not abnormal. Acute esophagitis and acute splenitis were noted, with myocardial fibrosis and moderate toxic changes in the other viscera. There was moderate generalized arteriosclerosis.

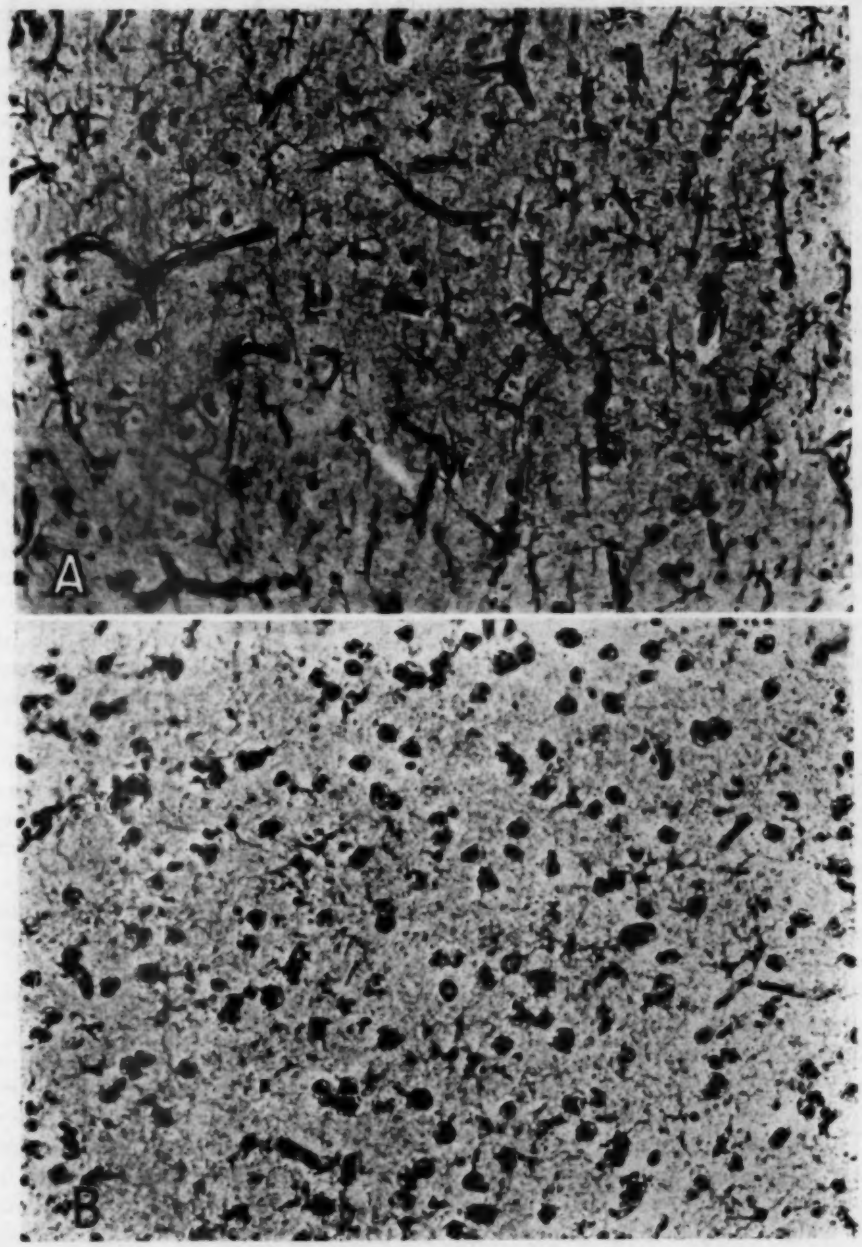
The brain weighed 1,210 Gm. Except for flattening of the gyri and narrowing of the sulci, no gross abnormalities could be found. Sections were taken from numerous areas of the cerebral cortex, from the hypothalamus, the brain stem, the pons, the medulla oblongata, and the cerebellum, from the upper cervical region of the spinal cord and from the peripheral nerves. Sections were stained with cresyl violet, with the Loyez myelin sheath and the Alzheimer-Mann methods and impregnated with silver by the Gross-Bielschowsky method and the methods of Bodian and Hortega.

Abnormalities of the nerve cells and the glia were found. In cresyl violet preparations of the cerebral cortex under low power magnification there was disclosed a diffuse disturbance in the cytoarchitecture of the gray matter. The lamellar arrangement was no longer clearly defined owing to loss of nerve cells and to increase in the number of glial elements, particularly of oligodendroglial and microglial nuclei. These alterations were present throughout the cerebral cortex without predilection for any special cortical zone or layer.

The nerve cell changes were of a degenerative nature. The affected cells showed diffuse chromatolysis and pronounced pyknosis. Some of the cells were entirely devoid of Nissl bodies; their protoplasm remained practically unstained. In some areas there were to be seen remnants of nerve cells only—so-called cell ghosts or shadows. Occasionally there could be found nerve cells with some swelling of their cytoplasm; the Nissl bodies were at the margins of the cytoplasm, and the nucleus was eccentrically placed.

Many of the ganglion cells revealed the severe type of degeneration of Nissl, with marked liquefaction of both the cytoplasm and the nucleus—rather complete disintegration of the cell. Only a few cells showed fatty degeneration. Fatty products were present only in limited amounts in the perivascular spaces and in some of the endothelial cells of the blood vessels. No trace of inflammation was to be seen either in the parenchyma or in the leptomeninges.

Paralleling these widespread degenerative nerve cell lesions were progressive and regressive glial changes. Enormous proliferation of microglia and oligodendroglia had occurred. Sections stained with the Hortega silver carbonate method disclosed an exceedingly cellular appearance of the cortical ribbon. This was mostly due to a marked increase in the number and the size of the microglia (*A* in figure). The majority of these cells were similar to those described as rod



A, diffuse proliferation and hypertrophy of the Hortega cells (microglia) in case 1. Hortega's silver carbonate impregnation; × 260.

B, proliferation and swelling of the oligodendroglia in case 1. Hortega's silver carbonate oligodendroglia impregnation method; × 260.

cells. They contained an elongated nucleus with scanty perinuclear protoplasm and showed long protoplasmic projections arising from opposite poles of the cell body. They usually paralleled the direction of the main dendrites of the pyramidal cells or accompanied the blood vessels; their main axes were at right angles to the surface of the cortex. Their location was predominantly cortical. In addition to their increase in number, the majority of the Hortega cells were markedly hypertrophied, the dendrites as well as the cell body. The prolongations of the dendrites were transformed into thick processes with sharp double contour and angular outline. Many cells were swollen and of coarsely reticulated structure. In some areas extremely elongated Hortega cells were abundant.

Oligodendroglia were also increased in number and swollen (*B* in figure). The nucleus was usually surrounded by an empty rounded space traversed by small septums connecting the outer border of the space with the nucleus. These swollen cells were exceedingly numerous in the deeper layers of the cortex and in the subcortex. They were gathered mostly about the nerve cells and around blood vessels, where they occasionally formed a wall of cells.

The reaction of the astrocytes was not uniform. Within the white matter, where they appeared to be dominant, they were afflicted with both progressive and regressive changes; the former consisted of hypertrophy of individual cells and slight increase in number. The regressive changes, which consisted of swelling and disintegration of the cell body and of the processes, were more pronounced—changes similar to those which have been described as clasmatodendrosis. Only relatively few glial cells showed ameboid degeneration. No gitter cell formation was to be seen.

Moderate cell changes were noted in sections of the hypothalamus, the brain stem, the pons and the cerebellum. In the latter there was a moderate degree of progressive and regressive glial change, especially marked within the white matter and in the dentate nuclei.

The myelin sheath preparations revealed no areas of primary destruction of myelin. Except for occasional rarefaction of sheaths in the white matter and a slight degree of incipient fragmentation of some of the sheaths, no pathologic changes were noted. No appreciable loss of myelin was visible in the course of the main pathways.

In sections from the lower part of the medulla and from the cervical and dorsal segments of the cord there was myelin loss in the white matter, especially in the tract of Goll of the posterior columns and in the crossed pyramidal tracts. In sections stained with cresyl violet these areas of demyelination were seen to contain moderately increased numbers of glial nuclei.

The sections from the peripheral nerves showed degeneration of the majority of the nerve fibrils. There were sausage-like swellings and beadings of the axis-cylinders. Some of the swollen fibers were many times the diameter of the normal fiber. Many axis-cylinders showed far advanced disintegration. They were fragmented into granular debris or had undergone a process of liquefaction with vacuolation. In some areas the degenerated fibers were separated by proliferated interfibrillary substance and an increased number of Schwann cells.

In summary, it appears that within the cortical tissue two main features characterized the pathologic process: widespread degeneration of nerve cells, which ranged from chromatolysis to cell shrinkage and shadow cell formation, and striking progressive and regressive glial changes. The cerebral lesions were accompanied by demyelination of the dorsal and lateral columns of the spinal cord and far advanced axonal degeneration of the peripheral nerves.

CASE 2

A 58 year old stuporous Negro woman was admitted to the Cincinnati General Hospital on Feb. 11, 1938. Over the years she had developed the habit of drinking large quantities of moonshine whisky. She was drunk the greater part of the time, and she ingested limited amounts of food, consisting principally of coffee. The consumption of alcoholic liquor had been further increased consequent to the death of her husband about two months before her admission. Apparent loss of weight had been observed in January 1938, and she became bedfast in late January, complaining of weakness. During this helpless period she continued to eat little or nothing, requesting whisky, which was supplied occasionally. On February 8 it was noted that her voice had become feeble and that she could not move herself about the bed. Sphincteric incontinence supervened. By February 10 she could only mumble unintelligibly and was extremely weak.

The exact duration of her alcoholism and malnutrition is not known, as her only intimate associate (husband) died in December 1937. Her history was given by a sister, who saw her infrequently, and by friends. She had borne one child.

She was stuporous and uncooperative, and her speech consisted of an unintelligible mumble. She was emaciated and weak. The chronic cutaneous lesions of pellagra were present on the backs of the hands and wrists, on the elbows, on the perineum, over the dorsal surfaces of the feet and over the lower parts of the legs, including the skin covering the patellas. The lesions were roughly symmetric on the two sides of the body and were sharply demarcated from normal skin. They were dry and scalelike. They were most severe in the perineal region, where the base was shiny, fiery red and raw.

The pupils were small. The breath was foul. The lips were cracked and crusted, and all of the buccal mucous membranes and gums were red and raw. The tongue was dry; the dorsum was covered with a thick coating, and the edges were shiny red, with atrophied papillae. The vaginal mucous membrane was fiery red. The musculature was flabby, and wasting was obvious in the small muscles of the feet and of the hands. The patient was generally weak. It was impossible to test sensation. She tried to remove herself from pinprick. The tendon reflexes were hypoactive, and there was no response to plantar stimulation.

During the six days that she lived in the hospital the pulse rate ranged between 95 and 118 beats per minute and the respirations between 22 and 40 per minute. The temperature was practically normal. The blood pressure ranged between 86 systolic and 60 diastolic and 116 systolic and 88 diastolic.

She experienced great difficulty in the swallowing even of liquids, and feeding by nasal tube had to be utilized. She vomited occasionally, necessitating the administration of fluid by vein and beneath the skin. One gram of nicotinic acid was administered in broken doses daily via nasal tube, and thiamine hydrochloride was given intravenously. The mucous membranes lost some of their redness with this therapy. Edema of the hands and feet developed on the fourth hospital day. She died on Feb. 16, 1938, the sixth hospital day.

The noteworthy laboratory findings were a negative Kahn test of the blood, red blood cell counts of 2.6, 2.8 and 2.5 millions, and hemoglobin contents of the blood of 11.4, 9 and 9.4 Gm. The serum protein on February 14 was 5.1 Gm. per hundred cubic centimeters. The blood urea nitrogen was 45 mg. per hundred cubic centimeters on February 11 and 43 mg. on February 16. The guaiac and benzidine tests for blood in the stool were positive on two occasions.

The clinical diagnosis was malnutrition, chronic alcoholism, pellagra and terminal uremia on the basis of arteriolonephrosclerosis.

Abnormal tissue changes besides those in the nervous system were confluent lobular pneumonia and acute bronchitis, atelectasis, marked fatty infiltration of the liver, marked hemosiderosis of the spleen, acute gastritis, mild chronic interstitial pancreatitis with diffuse fibrosis, chronic focal adrenalitis, chronic active vaginitis, generalized arteriosclerosis and arteriolosclerosis, diffuse and perivascular myocardial fibrosis, toxic myocardosis and nephrosis, and chronic cholecystitis.

The brain weighed 1,000 Gm. and disclosed diffuse cortical atrophy. There were advanced sclerosis and plaque formation of the vessels of the circle of Willis, also moderate dilatation of the ventricles.

Blocks taken from the frontal pole, the motor area and the occipital pole of the cerebral cortex, from the midbrain, the pons, the medulla oblongata and the cere-

bellum and from the spinal cord revealed the same changes as described in case 1 except for those in the spinal cord. In the sections taken from the cervical and thoracic segments of the spinal cord the number of anterior horn cells was slightly reduced. The remaining nerve cells disclosed fatty degeneration. A few shadow cells were to be seen among the cells of Clarke's column. The myelin sheath preparations did not disclose demyelination except for a slight degree of rarefaction of the posterior columns.

COMMENT

It seems to be agreed that diffuse degenerative changes of the nerve cells occur in the cerebral cortex of the person suffering from profound nutritional deficiency. Comparable findings have been reported in the nerve cells of the anterior horns and in Clarke's columns of the spinal cord. Myelin degeneration in the posterior and lateral columns of the spinal cord has been found inconstantly. Abnormality of peripheral nerves must be relatively common [1] in profound nutritional deficiency such as pellagra.

In experiments on starvation Ferraro and Roizin [2] found diffuse ischemic degeneration of the nerve cells widespread in the brains of cats. These changes in the nerve cells were associated with progressive and regressive glial changes.

In observations on the effects of hypoxia on the brain tissue in the wake of trauma, intoxications and asphyxia we have found the most constant abnormality to be lesions similar to those reported in these cases of nutritional deficiency. Obviously a feltwork of rod cells in the cerebral cortex is not a specific reaction, though it is seen most regularly in dementia paralytica. Morel and de Montmollin [3] recorded nerve cell change and rod cell formation in a particular clinical form of chronic alcoholism (pseudoparesis) and also in alcoholic pellagrous psychosis. In their experience and in ours one may or may not observe the overt cutaneous lesions diagnostic of pellagra in severe and chronic addiction to alcohol; nevertheless the changes in the cerebral cortex may be entirely comparable.

SUMMARY

Widespread changes in the cerebral cortex, the spinal cord and the peripheral nerves may be found in the nutritional deficiency associated with alcoholism. The cortical changes may include extensive formation of rod cells (hypertrophied microglia of Hortega).

1. Aring, C. D.; Bean, W. B.; Roseman, E.; Rosenbaum, M., and Spies, T. D.: Arch. Neurol. & Psychiat. **45**:772, 1941. Lewy, F. H.; Spies, T. D., and Aring, C. D.: Am. J. M. Sc. **199**:840, 1940.
2. Ferraro, A., and Roizin, L.: J. Neuropath. & Exper. Neurol. **1**:81, 1942.
3. Morel, F., and de Montmollin, R.: Monatschr. f. Psychiat. u. Neurol. **102**:210, 1940.

DIVERTICULA OF THE COLON IN RATS FED A HIGH FAT DIET

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Two outstanding features of diverticula of the colon are (1) their previously supposed occurrence only in man and (2) their restriction to the latter half of the normal life span. Up to the present there are no reports of diverticulosis in the literature except those of diverticulosis in man, and for that reason the diverticula which have been found in 3 rats which were fed a high fat diet for ninety to one hundred and eleven weeks are being reported.

Diverticulosis of the colon was found in 5.2 per cent of the 1,925 necropsies at the Mayo Clinic between 1924 and 1928. Only once was the condition found in a person under 40 years of age. These statistics are supported by roentgen examinations at the Mayo Clinic and by autopsies at the Cook County Hospital, Chicago. Diverticula occur most frequently on the sigmoid part of the colon, but they occur also on other parts and show an increasing tendency to occur on the lower part.[1]

The location of the diverticula has received much attention because of the general belief that the anatomy of the region is a highly important factor in their occurrence, as for instance the relationship of the blood vessels, the mesentery and the epiploic appendages to the layers of the wall, which results in a weak area and a disposition toward herniation. Other causes which have been advanced are: congenital defect, debilitating disease, senility, chronic constipation, stasis of the veins in the wall and sclerotic changes in the mesenteric arteries.[2]

It is a striking fact that although diverticulosis is believed to be limited to man, no explanations which justify this observation have been advanced. Consequently the diverticula in 3 rats which were fed a high fat diet seem worthy of being reported, since the publication of such a report may lead to further investigation of the relation of diet to diverticulosis rather than toward an explanation solely on an anatomic basis as heretofore.

MATERIAL AND OBSERVATIONS

The rats which showed diverticula at autopsy were members of the third generation which had been fed this high fat diet beginning at the time of weaning. There were 8 rats in this F_3 generation. Five were killed at twenty-five weeks of age and showed no signs of diverticula. The other three were maintained on the diet for ninety weeks or more. (Rat 207 was maintained for ninety weeks, rat 208 for one hundred and seven weeks and rat 209 for one hundred and eleven weeks.) The rats of the F_1 generation, as well as the parents of the F_1

From the Department of Anatomy, University of Pennsylvania.

1. Kocour, E. J.: Am. J. Surg. **37**:433, 1937. Rankin, F., and Brown, P.: Surg., Gynec. & Obst. **50**:836, 1930. Dixon, C. F.; Deuterman, J. L., and Weber, H. M.: ibid. **66**:314, 1938.

2. Chapman, J.: Ann. Int. Med. **7**:1376, 1934. Boyd, W.: Surgical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 307.

generation, were killed at 45 to 50 weeks of age. They showed no diverticula. The composition of the diet used was as follows:

	Per Cent by Weight
Butter fat	55
Casein	25
Cod liver oil	7
Dry yeast	7
Salt mixture (Osborne and Mendel [2a])	6

Three of the diverticula are shown in figures 1 to 2 *C*. A photograph of one of them includes all the abdominal viscera. The segment possessing the diverticulum was removed from one colon before it was photographed (fig. 2 *A*). In each of the 3 rats a diverticulum was situated within about 2 cm. of the cecum, and in 1 rat (209) there was a second diverticulum (diverticulum B) about half-way between the beginning and the termination of the colon. Two photographs of diverticulum B of rat 209 are shown (figs. 2 *B* and *C*). Each one shows the relationship of the diverticulum to the intestine.

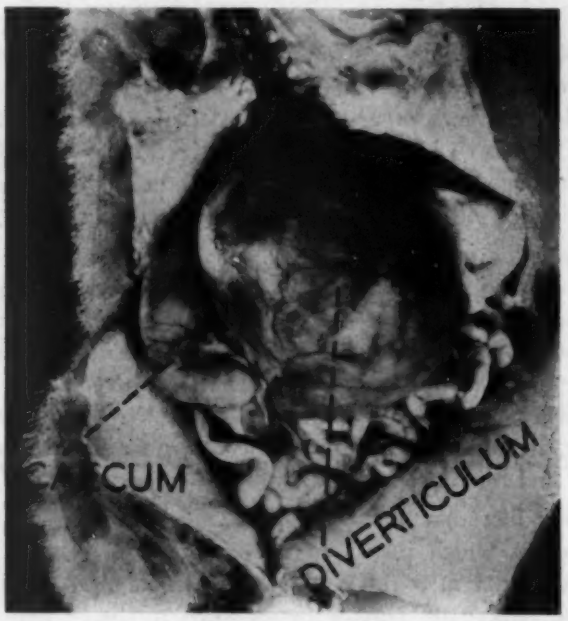

Fig. 1.—Photograph of opened abdomen of rat 207 showing diverticulum in situ. × 0.75.

The diverticulum shown in figure 1 (rat 207) is located on the colon 1.5 cm. beyond the cecum. It is a pouchlike diverticulum, almost spherical in form, measuring 46 mm. in length and 37 mm. in diameter. Its volume is 23 cu. cm. The diverticulum is clearly not a simple dilatation of the colon but a bulging outward of a part of the wall, resembling diverticula which occur on the human intestine. The connection between the diverticulum and the colon cannot be seen in the photograph, but the length of the portion of the colon involved by the diverticulum (in its final state of development) is 2.0 cm. The diverticulum is seen to be a development in the direction of the free border of the colon, not toward the mesentery. The blood vessels supplying this part of the colon have no clear relationship to the position of the diverticulum. The extensive size of the diverticulum brought it into contact with the parietal peritoneum, and an adhesion has developed between the diverticulum and the parietal peritoneum. No other pathologic condition was observed in the abdominal cavity.

The diverticulum found in rat 208 was situated about 2.0 cm. beyond the cecum. The gross appearance of this diverticulum was strikingly different from the one shown in figure 1. In rat 208 the diverticulum had developed and extended dorsally toward the mesocolon. The

2a. Osborne, T. G., and Mendel, L. B.: J. Biol. Chem. **34**:131, 1918.

wall of the diverticulum was relatively thick (3 to 6 mm.); this was due to proliferation and thickening of the mesentery of the small intestine, which adhered to the external surface of the diverticulum. The mucosal surface of this diverticulum was folded into elevations 1 to 2 mm. in height, in contrast to the grossly smooth mucosal surface of the previously described diverticulum (fig. 1). The loops of the small intestine were strongly adherent to the diverticulum and formed the outer layer, but there was no fistula between the diverticulum and the small intestine. This diverticulum had a volume of approximately 10 cc.

The rat identified as 209 had two diverticula of the colon. The larger of the two was about 1.5 cm. from the cecum (fig. 2*A*). It extended toward the free border of the colon and

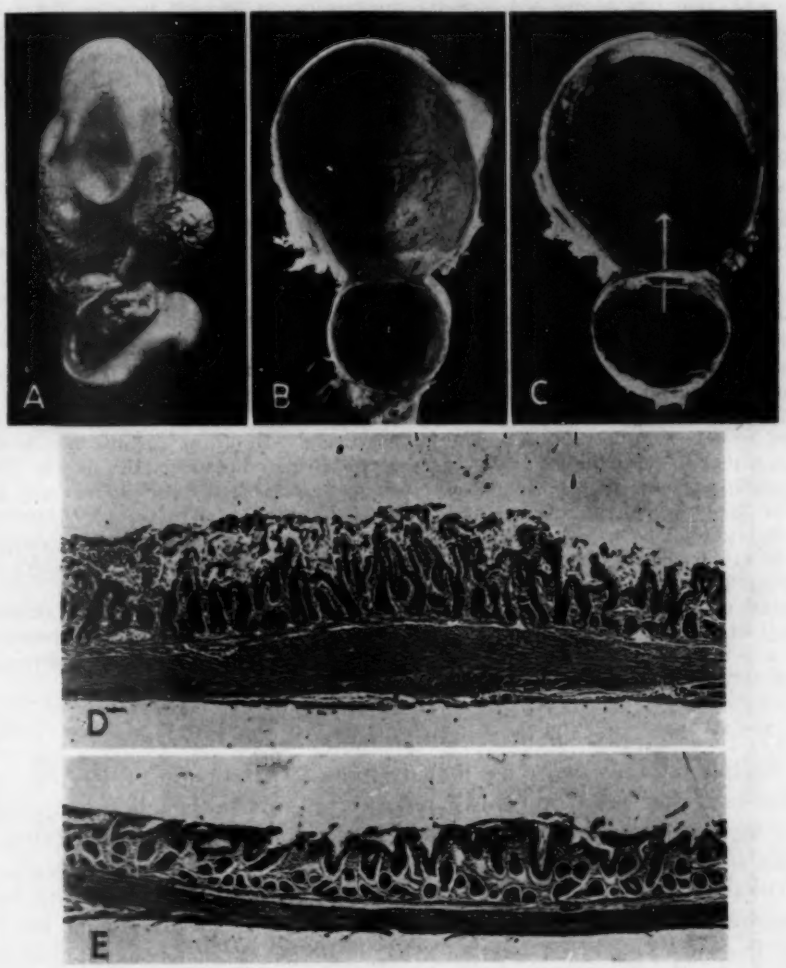

Fig. 2.—*A*, photograph of the isolated diverticulum A of rat 209. The cecum is also included. × 0.75.

B and *C*, two parts of diverticulum B of rat 209 including the adjacent colon. The arrow indicates the opening between the colon and the diverticulum. *B* shows one half of diverticulum B. × 1.5.

D, section of the wall of diverticulum A of rat 209 (see *A*). The muscle layer is very thick. The outer layer of muscle is cut in cross section. It is incomplete. Hematoxylin and eosin stain; × 25.

E, section of the wall of the diverticulum shown in figure 1. The muscle layer runs predominantly in one direction. Hematoxylin and eosin stain; × 25.

measured 45 mm. in length and 25 mm. in diameter. The volume was 18 cc. The external surface was smooth and almost free from adhesions. The distal part of the small intestine was, however, adherent to the diverticulum, and a fistula had developed between the ileum and the diverticulum. The wall of the diverticulum was thin as though distended by pressure of the contained fecal mass. There was no evident relationship between the site of entrance of the blood vessels which supplied the colon and the site of the diverticulum. The other diverticulum of this rat was situated nearly halfway between the beginning of the colon and the anus. It was a small spherical diverticulum which extended toward the free border of the colon and measured 20 mm. in diameter. Its volume was calculated to be 4.2 cc. Photographs are shown of this diverticulum after it had been opened by two incisions so arranged that each incision came just to the sides of the communicating opening between the colon and the diverticulum (see fig. *2 C*). The dimensions of this communication were 1.0 by 2.0 mm., and it was almost rectangular in shape. This area had, through distention, expansion and hyperplasia, increased to form the wall of the diverticulum. In figure *2 B* one half of this diverticulum is shown.

DESCRIPTION OF THE MICROSCOPIC STRUCTURE OF THE WALL OF THE DIVERTICULA

Segments of the wall of each diverticulum were removed, dehydrated, embedded, stained and studied microscopically. The segments were chosen with care so that they included the junction between the wall of the colon and the wall of the diverticulum. Some of the sections were stained with hematoxylin and eosin; other sections were stained for elastic fibers, with resorcin-fuchsin. Photomicrographs showing the layers of the walls of the diverticula are presented in figures *2 D* and *E*.

The microscopic sections show that the diverticula are not herniations of the mucous and submucous layers of the colon through the muscle layers, as in acquired diverticulosis in man.[3] Instead the wall has a thick muscular component. In certain areas it is slightly thinner than that found in the wall of the colon, but in diverticulum B of rat 209 the muscle layer is thicker than that in the adjacent colon. In the two largest diverticula the muscle fibers run at nearly right angles to each other and give the appearance of an inner circular and an outer longitudinal layer of muscle. The muscle layer of diverticulum B of rat 209, however, has a single direction. The mucous membrane of the diverticula is not especially compressed or flattened from internal pressure except in the case of diverticulum B of rat 209, which is much distended and thinned.

The sections stained with resorcin-fuchsin show a considerable amount of elastic tissue in the wall of the colon and the diverticulum—especially in the vicinity of the blood vessels. Old rats which received a balanced diet were compared with these 3 rats with diverticula as to the amount of elastic tissue in the colon, but no real difference was found.

COMMENT

The finding of diverticula on the intestines of 3 rats is of interest because diverticula have not been previously reported in rats, although the diverticulum is one of the most common abnormalities of the colon of the human being. The diverticula observed in these rats resembled in appearance the diverticula of the human colon, and seems to be the result of high pressure within the intestine acting against a specific, limited area of the wall. Such diverticula are clearly different from the epithelial overgrowths observed by Lubbock, Thomson and Garry[4] in rats which were fed a low grade "human" diet.

A search of the literature for reports on the occurrence of diverticula in the rat was fruitless, and no reference was found to diverticula except in man. Since it was possible that diverticula were being found but not reported, two of the most likely sources of information were approached for unpublished reports. E. J. Farris, of the Wistar Institute of Anatomy and Biology, in a personal communication offers the information that there are no records of diverticula having been observed there. Their records cover a period of approximately thirty years,

3. Butler, P. F., and Ritvo, M.: Boston J. Med. & Surg. **192**:705, 1925.

4. Lubbock, D. M.; Thomson, W., and Garry, R. C.: Brit. M. J. **1**:1252, 1937.

during which rats reared on a stock diet were examined post mortem at various ages. Their records include fifty generations of the wild Norway rat which were killed and examined between the ages of 18 months and 2 years.

The other source of information which was drawn on for data concerning unpublished observations was the Cornell University Laboratory of Animal Nutrition. Dr. C. M. McCay and his co-workers have been interested for the past decade in the longevity of rats fed a restricted diet. In a personal communication from Dr. McCay it was learned that no diverticula had been observed in any of their rats. Some of the rats in the experiments conducted by Dr. McCay and his co-workers lived as long as thirteen hundred days. The diet fed was a balanced diet that was restricted in amounts for a varying length of time, as long as one thousand days for some rats, and then increased to normal.[5] In view of these data it would seem that the rat is immune to diverticulosis of the intestine when fed a balanced diet, even when it reaches an age beyond that equivalent to a human being 109 years of age.

Since diverticula have been neither reported nor observed in rats which were fed a balanced artificial or stock diet, it is necessary to inquire regarding their occurrence on the feeding of unbalanced diets, such as the high fat diet which was fed in these experiments. Again the literature contributes little information. A high fat diet of approximately the same composition as that used in these experiments was fed to rats for periods up to four hundred days in a study of the effects on the growth of the body.[6] Unfortunately the autopsy observations on these rats have not been reported, but from the growth curves it is unlikely that diverticula were present.

The possibility that the diverticula are due to defective germ plasm seems remote. The basis for this opinion is the fact that the Wistar strain has not shown any such single anomaly of the colon previously. The "stub strain" of the Wistar colony shows certain defects of the colon, such as atresia, but they are accompanied by massive defects of the pelvis so that they appear to be secondary. Such rats are always nonviable.[7]

Diverticula of the rat resemble in several respects diverticula of man. In respect to age incidence, diverticula of the colon are rarely reported in man before the fortieth year, and in these rats they were not found until the ninetieth week, which is equivalent to the fifty-second year in the life span of the human being. Diverticula occur most frequently on the sigmoid (pelvic) part, the most tortuous part, of the human colon.[1] They occur on the most tortuous part also of the rat colon, but this part is near the cecum. They extend either toward the free, unattached border of the colon or toward the mesentery, and resemble in that respect, also, the diverticula of the human intestine.

In none of the cases was there anything about the pattern or the size of the blood vessels at the site of the diverticulum to indicate that they might play a role in the origin of the anomaly. As Chapman pointed out, the arteries divide into small branches before they penetrate deeply into the wall, and thus they do not weaken it greatly. The diverticula which developed toward the unattached surface were much larger than the ones which developed toward the mesentery. This may have been due to a lack of adjacent restraining structures ventrally, compared with the presence of the mesocolon of the dorsal part of the abdominal cavity.

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5. McCay, C. M.; Maynard, L. A.; Sperling, G., and Barnes, L. L.: J. Nutrition **18**:1, 1939.
6. Levine, H., and Smith, A. H.: J. Biol. Chem. **72**:223, 1927.
7. Ratcliffe, H. L., and King, H. D.: Anat. Rec. **81**:283, 1941.

The presence of a muscle layer in the wall of the diverticulum seems to be due to the fact that there may have been no weak spot in the muscle layer, such as occurs when large blood vessels directly penetrate the muscle layer of the tract.[8] Even though muscle formed a complete layer of the wall of diverticulum, the possibility still remains that atrophic changes occurring with age in the submucous coat after stasis are primarily the cause of diverticula, as postulated by Chapman. However, the microscopic sections show an abundance of yellow elastic fibers in the wall of the colon as well as in the wall of the diverticula. It seems that if atrophic changes of the submucous layer occur, they do not consist primarily in a decrease in elastic fibers. Epithelial overgrowths such as were observed by Lubbock, Thomson and Garry were not found in any of these rats.

SUMMARY

One or more diverticula of the colon developed in each of 3 rats fed a high fat diet for ninety weeks or longer. The regularity of their position and the similarity in their walls seem to indicate a uniform response to given conditions.

8. Wierda, J. L.: Anat. Rec. **79**:109, 1941.

General Reviews

HODGKIN'S DISEASE

THE INCIDENCE, DISTRIBUTION, NATURE AND POSSIBLE SIGNIFICANCE OF THE LYMPHOGRANULOMATOUS LESIONS IN THE BONE MARROW
A REVIEW WITH ORIGINAL DATA

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That Hodgkin's lymphogranulomatosis is not a disease of lymph nodes exclusively, and indeed frequently not even primarily, has become apparent as the natural history of the disease has been studied further. The synonym "granulomatosa maligna" (Benda [1]), or "malignant granuloma," was coined (and has attained wide usage in French, Italian and German speaking countries, with some justification) to dispel the notion of a close relationship of this disease to the lymphatic system as implied in the name "lymphogranuloma." The osseous system has shared increased attention with the lungs and the alimentary tract among the nonlymphatic organs. The lesions in the bones are of interest to the diagnostician, the roentgenologist, the surgeon and the pathologist alike.

The osseous lesions in lymphogranulomatosis were first described by Benda,[1] in 1904, although changes which were possibly examples of this disease had previously been presented by Hammer,[2] in 1894, and by others. The uncertainty about these earlier cases arises from the fact that a clear histologic description of this disease was not made until later—by Sternberg [3] in 1898 and by Reed [4] in 1902 (Jones [5]).

Between 1904 and 1920 osseous involvement in Hodgkin's disease was described as an incidental feature in numerous papers which dealt with general aspects of the disease. In 1920 the first paper dealing exclusively with the osseous lesions from the point of view of pathology was published by Askanazy [6]; it was followed in 1922 by the first paper dealing with the roentgenologic features.[7] From this time papers began to appear frequently so that within a period of two decades (1922-1942) no less than ninety-two titles of papers dealing primarily with the skeletal changes were recorded in the *Index Medicus* and the *Quarterly Cumulative Index Medicus*. This figure does not include numerous additional papers in which lesions of bones were described incidental to involvement of the spinal cord or papers in which the osseous lesions were of secondary interest. Many of the ninety-two papers present only 1 or 2 cases, but others report on large series which number in the clinical studies up to 66 cases, reported by Dresser and Spencer,[8] and in the necropsy studies up to 28 cases, described by Stephani.[9]

From the Department of Pathology of the University of Chicago.

1. Benda, C.: Centralbl. f. allg. Path. u. path. Anat. **15**:542, 1904.
2. Hammer: Virchows Arch. f. path. Anat. **137**:280, 1894.
3. Sternberg, C.: Ztschr. f. Heilk. **19**:21, 1898.
4. Reed, D. M.: Johns Hopkins Hosp. Rep. **10**:133, 1902.
5. Jones, G. W.: Ann. M. Hist. **2**:471, 1940.
6. Askanazy, M.: Centralbl. f. allg. Path. u. path. Anat. **31**:557, 1920.
7. Grossmann, A., and Weis-Ostborn, W.: Fortschr. a. d. Geb. d. Röntgenstrahlen **29**:569, 1922.
8. Dresser, R., and Spencer, J.: Am. J. Roentgenol. **36**:809, 1936.
9. Stephani, H.: Virchows Arch. f. path. Anat. **300**:495, 1937.

PATHOGENESIS

The skeleton may become involved in Hodgkin's disease in any of three ways, namely, by direct invasion, perhaps mediated through the lymphatics sometimes, by conveyance in the blood stream to the site or by primary origin in the bones. These modes, especially the first two, may concur in some cases.

Direct invasion takes place from contiguous lymphogranulomatous masses which are usually located in lymph nodes. Hence, osseous lesions produced by direct invasion are most common in those parts of the skeleton which are in anatomic relation to the large groups of lymph nodes. These bones include the spine, the sternum, the pelvis and the inner ends of the clavicles and ribs. The skeletal lesions which result from this method of attack are destructive, especially of bone cortex, and often they become large. Consequently they may produce symptoms, and for this reason, as well as because of their size and their location on the surfaces of bones, they are often recognized during life and at necropsy. Most of the papers on Hodgkin's disease of bone deal entirely or predominantly with lesions of this genesis, and the impression is obtained from the literature that it is the commonest genesis for the skeletal lesions found in this disease.

In the second form of lymphogranulomatous involvement of the skeleton, scattered lesions are found in various bones, including those not in proximity to lymph nodes or to the other commonly diseased soft tissue. They may be regarded as hematogenous metastases caused by emboli either of cells or of a living agent, depending on the views held by the observer regarding the etiology of this disease. These lesions, also, may become large enough to produce symptoms which lead to their disclosure. Often, however, they remain small, and they may be unrecognized unless they are sought. It is believed that all of the hematogenous skeletal lesions begin in the marrow and that only after they have enlarged and caused changes in the bone are they revealed by roentgenograms. It is well known that patients with large lesions confined to the marrow may have negative roentgenograms. Tetzner,[10] Herscher [11] and Reisner and Brada,[12] among others, have described such cases. The smaller lesions of marrow are even harder to visualize. Probably the great majority cannot be revealed by present roentgenographic technics and are unrecognized.

The principal sites for the hematogenous lesions are in the vertebrae, the femurs, the skull, the sternum, the ribs and the pelvis. It has been pointed out that these lesions, like metastases of carcinoma in the skeleton, have the distribution of the red marrow (Uehlinger [13]). It is emphasized in the third part of this paper that this distribution is also that of the reticuloendothelial cells in the marrow and that for several reasons this disease should be regarded as a disease of that system as a whole.

The third method of skeletal involvement is stated to be by origin of the disease in the marrow. While this is theoretically possible, especially to those who consider Hodgkin's disease to be a neoplastic disease of reticulum cells, which are abundant in the marrow, its occurrence has not been proved beyond doubt. No case has been reported in which at necropsy the lesions were found to be confined to the skeleton. Two cases have been reported in which lesions of bones but none of lymph nodes were found. Krumbhaar [14] reported such a case with involvement of the marrow and

10. Tetzner, E.: Frankfurt. Ztschr. f. Path. **42**:545, 1932.
11. Herscher, H.: Am. J. Roentgenol. **35**:73, 1936.
12. Reisner, A., and Brada, H.: Röntgenpraxis **5**:182, 1933.
13. Uehlinger, E.: Virchows Arch. f. path. Anat. **288**:36, 1933.
14. Krumbhaar, E. B.: Am. J. M. Sc. **182**:764, 1931.

the spleen, and Livingston [15] and Herscher [11] reported another with involvement of the bones and the liver. On the other hand, many cases have been reported in which the first clinical manifestation of the disease was from a bone lesion, demonstrable involvement of lymph nodes or other viscera occurring only later.[16] These cases do not prove origin in the bones because it is possible that deep lymph nodes or other tissues were previously involved, but they give strong support to such an idea.

MORPHOLOGY

The bone lesions, regardless of genesis, may be osteolytic, osteogenic or mixed. The former may lead to pathologic fracture [17] and, in the case of the spine, to collapse of vertebrae,[18] sometimes with gibbus.[19] Sequestration of bone has been described. Destructive lesions which resembled multiple myeloma have been reported.[20]

The bone-stimulating forms may produce osteophytes,[17a] osteopetrosis [21] or more widespread osteosclerosis.[22] Endosteal [13] and periosteal forms [23] have been described.

The lesions of bones may resemble at times primary tumors, metastatic tumors and osteomyelitis. To the naked eye and on the roentgenograms there is nothing which reliably identifies them as Hodgkin's lymphogranuloma. This can be done only on microscopic examination.

Lymphogranulomatous lesions in the marrow may be of microscopic size. Frequently, however, they are visible as scattered nodular areas which are yellowish white or grayish white and which may stand out distinctly because the surrounding marrow is often darker than normal (figure). In other cases large areas of marrow may be replaced by a yellowish or grayish tissue, the bone itself showing little or no change (*B* in figure).

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15. Livingston, S. K.: J. Bone & Joint Surg. **17**:189, 1935.

16. (*a*) Montgomery, A. H.: Ann. Surg. **87**:755, 1928. (*b*) Blount, W. P.: J. Bone & Joint Surg. **11**:761, 1929. (*c*) Friedrich, H.: Fortschr. a. d. Geb. d. Röntgenstrahlen **41**: 206, 1930. (*d*) Teenstra, C. P. H.: Nederl. tijdschr. v. geneesk. **82**:391, 1938. (*e*) Herskovitz, E.: Röntgenpraxis **10**:114, 1938. (*f*) Lieberman, H. S.: J. Bone & Joint Surg. **20**:1039, 1938. (*g*) Ledoux-Lebard, R.: Marchand, J. H., and Lefebvre, J.: Bull. et mém. Soc. d'électro-radiol. méd. de France **27**:150, 1939. (*h*) Pellé, and Massot, H.: Bull. et mém. Soc. méd. d. hôp. de Paris **55**:372, 1939. (*i*) Paraf, J.; Fischgold, H.; Abaza, A., and Lewi, S.: Sang **13**:797, 1939. (*j*) Heider, K.: Ztschr. f. klin. Med. **136**:240, 1939. (*k*) Stäudtner, F.: Deutsche med. Wchnschr. **59**:1564, 1933. (*l*) Lockwood, I. H.; Johnson, E. J., and Narr, F. C.: Radiology **14**:445, 1930. (*m*) Pechel: Deutsche med. Wchnschr. **59**:114, 1933. (*n*) Dresser, R.: Am. J. Roentgenol. **15**:525, 1936. (*o*) Dresser and Spencer.[8]

17. (*a*) Beitzke: Verhandl. d. deutsch. path. Gesellsch. **13**:224, 1909. (*b*) Barron, M.: Arch. Path. **2**:659, 1926.

18. (*a*) Robin, N. H.: Am. J. Roentgenol. **14**:251, 1925. (*b*) Grudzinski, Z.: J. de radiol. et d'électrol. **12**:269, 1928. (*c*) Lemierre, A., and Augier, P.: Ann. d'anat. path. **8**:916, 1931. (*d*) Bodechtel, G., and Guizetti, H. U.: Ztschr. f. d. ges. Neurol. u. Psychiat. **149**: 191, 1933. (*e*) Bietzke, H.: Virchows Arch. f. path. Anat. **296**:358, 1935. (*f*) Hippe, H., and Hähle, K.: Röntgenpraxis **9**:116, 1937. (*g*) Herskovitz.[16e] (*h*) Heider.[16j]

19. Blount.[16b] Barron.[17b]

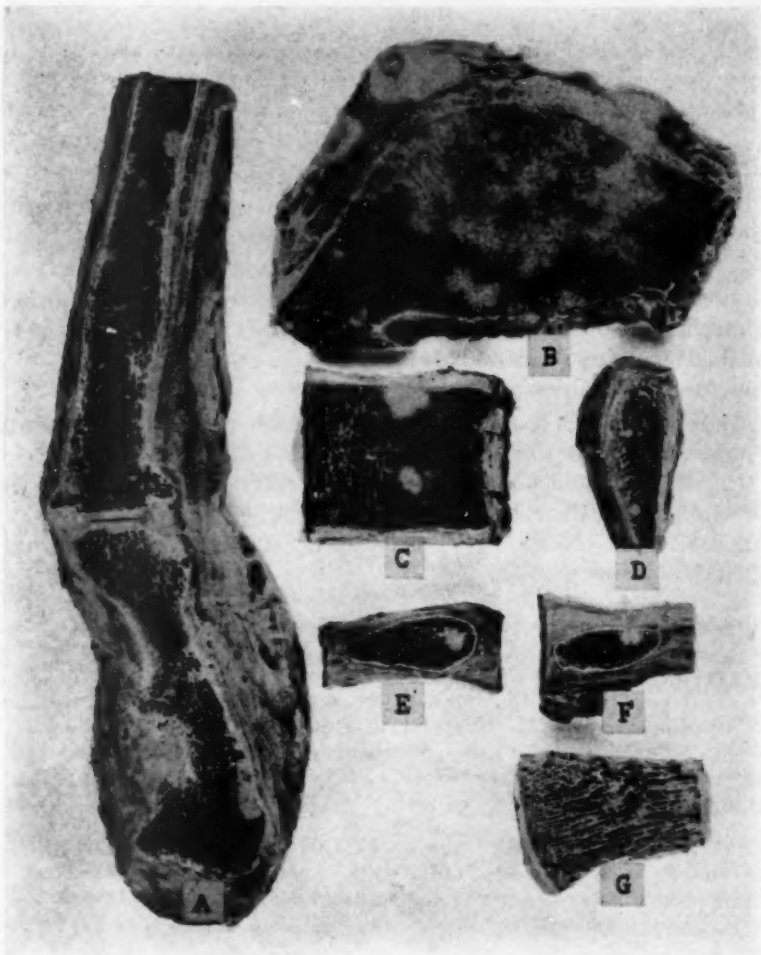
20. Brandt, M.: Frankfurt. Ztschr. f. Path. **46**:508, 1934. Herscher.[11] Lockwood and others.[16l]

21. (*a*) Camplani, M.: Radiol. med. **22**:39, 1935. (*b*) Herscher, H., and Stein, J. J.: Am. J. Roentgenol. **43**:74, 1940.

22. Hultén, O.: Acta radiol. **8**:245, 1927. Kuckuck, W.: Röntgenpraxis **3**:190, 1931. Bresci, L.: Clinica **6**:670, 1940. Bodechtel and Guizetti.[18d]

23. (*a*) Morrison, M. C.: Canad. M. A. J. **34**:393, 1936. (*b*) Uehlinger.[13]

Histologically, the lymphogranulomatous tissue in the marrow shows the same range of variation which such tissue in other organs and tissues exhibits. The same pleomorphism of cell type is present. Reticulum cells, mononuclear and multinuclear giant cells, eosinophilic leukocytes and myelocytes, neutrophilic polymorphonuclear leukocytes, lymphocytes, plasma cells, fibroblasts and fibrous tissue may be present in various combinations. Consequently the tissue may be cell rich



Lymphogranulomatous lesions in the marrow in a case of Hodgkin's disease. *A* is the manubrium and half of the sterum. *B* shows a transection through the fourth lumbar vertebral body, and *C*, a middorsal vertebral body. *D* is the right iliac crest, and *E* and *F* are the right third and second ribs. *G* is the only bone without a lesion. It is the left first metatarsal.

or scirrhous. When it is scirrhous, it may show considerable edema in addition to the paucity of cells.

INCIDENCE AND SITES REPORTED IN THE LITERATURE

The incidence of lymphogranulomatous lesions in the skeleton as reported in the literature shows wide variations, which seem to depend on the different methods

of study. Most of the reports represent clinical investigations. Some of the clinical reports deal with unusual cases, especially those in which the spine or the sternum was involved. Others are concerned with the osseous lesions which were found in series of cases. The data from a representative group of such reports, all based on over 10 cases, are assembled in table 1. Most of these papers were not primarily concerned with osseous lesions. Here it is seen that the percentage of the cases which showed involvement of bones varies from 0 to 15.7 and that the average for the entire group is 8.3. Among 2,006 cases there were 166 with lesions of bones. In reviewing these papers one gains the impression that the great

TABLE 1.—*Incidence of Lesions of Bone in Cases of Hodgkin's Disease as Reported in Clinical Studies*

Author *	Cases	Number with Lesions of Bone	Percentage with Lesions of Bone	City
Baker and Mann	65	0	0	London, England
Baldridge and Awe	46	6	13.0	Iowa City
Belot and co-workers	33	4	12.1	Paris, France
Burger and Lehman	54	4	7.4	Charlottesville, Va.
Burnam	173	2	1.1	Baltimore
Craver and Copeland [21a]	172	27	15.7	New York
Dresser	244	20	8.2	Boston
Fraser and Mekie	17	1	5.9	Edinburgh, Scotland
Goldman [21b]	212	14	6.6	New York
Haden and Burns	47	0	0	Cleveland
Kremser	51	2	3.9	Hamburg, Germany
Krueger and Meyer [21c]	60	9	15.0	Madison, Wis.
Mills and Pritchard	20	2	10	Montreal, Canada
O'Brien	60	5	8.3	Boston
Peirce and co-workers	214	11	5.3	Ann Arbor, Mich
Raagaard	19	2	10.5	(Denmark)
Reisner and Brada [12]	95	7	7.4	Frankfurt, Germany
Santagati	60	4	6.6	Milan, Italy
Schenck	107	8	7.5	New York
Vieta and co-workers [25]	257	38	14.8	New York
Totals	2,006	166	8.3	

* Complete references for all authors not mentioned in footnotes to the text of this article are added in alphabetical order here:

Baker, C., and Mann, W. N.: Lancet **1**: 23, 1940.
Baldridge, C. W., and Awe, C. D.: Arch. Int. Med. **45**: 161, 1930.
Belot, J.; Nahan, L., and Kimpel, J.: J. de radiol. et d'électrol. **12**: 257, 1928.
Burger, R. E., and Lehman, E. P.: Arch. Surg. **43**: 839, 1941.
Burnam, C. F.: J. A. M. A. **87**: 1445, 1926.
Dresser, R.: Strahlentherapie **41**: 401, 1931.
Fraser, J., and Mekie, E. C.: Edinburgh M. J **40**: 445, 1933.
Haden, R. L., and Burns, J. T.: Cleveland Clin. Quart. **9**: 144, 1942.
Kremser, K.: Röntgenpraxis **2**: 906, 1930.
Mills, E. S., and Pritchard, J. E.: Canad. M. A. J. **33**: 50, 1935.
O'Brien, F. W.: Am. J. Roentgenol. **46**: 80, 1941.
Peirce, C. B.; Jacox, H. W., and Hildeth, R. C.: Am. J. Roentgenol. **36**: 145, 1936.
Raagaard, O.: Ugesk. f. læger **98**: 759, 1936.
Santagati, F.: Radiol. med. **25**: 711, 1938.
Schenck, S. G.: New York State J. Med. **37**: 27, 1937.

majority of the lesions of bones were of the type caused by direct extension into the skeleton. Invariably the principal sites are given as the vertebrae, the pelvis, the sternum and the ribs.

Another group of papers deals with the bone lesions which were found at necropsy in series of cases. Here again there is great variation in the incidence of skeletal lesions, depending apparently on the thoroughness of the examination of the skeleton. The data assembled from a few reports in the literature are given in table 2. Among 547 necropsies in cases of Hodgkin's disease 155 disclosed lesions of bones (28.3 per cent). The incidence was low when the skeleton was examined only as indicated by the clinical and roentgenographic findings, and it was high when the examination of bones was routine and thorough. Thus, Stephani [9] stated that the spine and one femur were always examined, whereas

Symmers [24] emphasized that the skeleton was examined thoroughly because of special interest.

An analysis of the sites involved in this disease is given in table 3. The cases used in preparing this table were those reported in the ninety-two papers previously mentioned as dealing primarily with osseous lymphogranuloma. Duplication of cases was avoided as far as possible. The only large series of cases included were those of Uehlinger,[13] Dresser and Spencer [8] and Vieta, Friedell and Craver.[25] All other papers dealt with less than 10 cases each. The table shows a striking differ-

TABLE 2.—*Incidence of Skeletal Involvement in Cases of Hodgkin's Disease as Reported for Series of Necropsies*

Author *	Cases	Number with Lesions of Bone	Percentage with Lesions of Bone	City
Barron [17b]	24	4	16.6	Minneapolis
Krueger and Meyer [21c]	16	5	31.3	Madison, Wis.
Rolleston	39	19	48.7	London, England
Stephani [9]	70	28	40.0	Berlin, Germany
Supino	20	1	5.0	Milan, Italy
Symmers [24]	14	7	50.0	New York
Terplan and Mittelbach	29	7	24.1	Prague, Czechoslovakia
Tetzner [16]	10	9	90.0	Dresden, Germany
Uddströmer	192	28	14.6	(Sweden)
Uehlinger [13]	50	15	30.0	Zurich, Switzerland
Vasiliu and Goia	13	1	7.7	Cluj, Rumania
Vieta and co-workers [25]	47	23	48.9	New York
Webster	11	0	0.0	Baltimore
Werthemann	12	8	66.7	Basel, Switzerland
Totals	547	155	28.3	

* Complete references for all authors not mentioned in footnotes to the text of this article are added in alphabetical order here:
Rolleston, H.: Lancet **2**:1209, 1925.
Supino, L.: Tumori **8**:274, 1934.
Terplan, K., and Mittelbach, M.: Virchows Arch. f. path. Anat. **271**:759, 1929.
Uddströmer, M.: Acta tubere. Scandinav., 1934, supp. 1, p. 1.
Vasiliu, T., and Goia, I.: Ann. d'anat. path. **4**:33. 1927.
Webster, L. T.: Johns Hopkins Hosp. Rep. **20**:251, 1921.
Werthemann, A.: Schweiz. med. Wchnschr. **99**:868, 1938.

TABLE 3.—*Sites of Osseous Lesions in Lymphogranulomatosis Collected from the Literature (269 Cases in 92 Papers)*

	Cases		Cases
Vertebra	196	Clavicle	11
Pelvis	81	Scapula	9
Rib	67	Tibia	8
Femur	43	Mandible	1
Sternum	42	Maxilla	1
Skull	24	Radius	1
Humerus	15	Os calcis	1

ence in incidence of involvement of the various bones. It is to be emphasized again that it concerns the incidence and the distribution of lesions which were of clinical significance and not the absolute incidence and distribution.

Frequently more than one bone was involved in the cases reported. The true frequency of multiple osseous involvement, however, is not apparent from these data because most of the papers were reports by roentgenologists, and they were often published before the disease had run its full course.

The statement is often made that lymphogranulomatous involvement of the skeleton is a late manifestation of the disease. This idea appears to be unwarranted.

24. Symmers, D.: Am. J. M. Sc. **167**:157 and 313, 1924.
25. Vieta, J. O.; Friedell, H. L., and Craver, L. F.: Radiology **39**:1, 1942.

according to the careful study of Vieta, Friedell and Craver.[25] They stated that in only 31 per cent of their patients did the bone lesions first appear during the final one third of the disease span.

From these reports the following facts have been established: that in Hodgkin's disease the lesions in bone may involve practically any part of the skeleton, although there are sites of predilection; that bone at times seems to be the primary site of origin of the disease; that the lesions may show great variation in their gross morphologic characteristics and clinical effects, mimicking at times the lesions of bone, both primary and secondary, in many other diseases and that they are common, although there is lack of agreement as to precisely how common.

ORIGINAL DATA ON THE INCIDENCE AND THE SITES OF LYMPHOGRANULOMATOUS LESIONS OF THE MARROW

Original data on the frequency with which lymphogranulomatous lesions of the marrow occurs in cases of Hodgkin's disease and on the sites are presented in this section of the paper, and the significance of the lesions is discussed from several points of view. The other types of lesions which are found in the marrow in this disease are not discussed here but will be dealt with in another paper.

From the reports in the literature it is difficult to determine the incidence of lymphogranulomatous lesions of marrow as distinguished from those of bone. Both Simonds [26] and Wallhauser [27] in their reviews emphasized this point. Ziegler [28] stated that lesions of marrow are present in 30 to 40 per cent, and Symmers,[24] Ewing [29] and Medlar [30] thought that involvement of marrow is the usual thing, although they gave no supporting data.

In this study the incidence of lymphogranulomatous lesions of the marrow was determined by microscopic examination of sections prepared from small pieces of bone removed from various parts of the skeleton at necropsy in 14 consecutive cases. The bones sampled were selected because of their accessibility and not because of preexisting information from roentgenographic or clinical studies that lesions were present. It was not the practice to remove large pieces of bone and then select for microscopic study that portion of each which showed lesions. This paper, therefore, is not concerned primarily with those osseous lesions of lymphogranuloma which were recognized clinically and by roentgenograms during life or with those which were found at necropsy because they were massive. Numerous papers have dealt with the incidence and the significance of such frank lesions of the bones, and have established the point that they are present in nearly 10 per cent of persons who have this disease (table 1). While such surveys deal with lesions of clinical importance, they are not adequate for the study of the true distribution of the disease.

At necropsy small pieces were removed from three to nine different bones in each case, at the sites shown in table 4. They were fixed in 4 per cent solution of formaldehyde or in Zenker's solution, decalcified, embedded in celloidin (a concentrated preparation of pyroxylin) or in paraffin, cut, and stained with hematoxylin-eosin and occasionally with other stains. The size of each section was such that it could usually be mounted on the ordinary slide, which is 1 inch (2.5 cm.) wide.

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26. Simonds, J. P.: Arch. Path. **1**:394, 1926.
27. Wallhauser, A.: Arch. Path. **16**:522 and 672, 1933.
28. Ziegler, K.: Die Hodgkinsche Krankheit, Jena, Gustav Fischer, 1911.
29. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.
30. Medlar, E. M.: Am. J. Path. **7**:499, 1931.

The incidence of lymphogranulomatous lesions of the marrow in sections of 62 bones obtained at 14 necropsies, together with the sites of the sampling, is presented in table 4. Lymphogranulomatous foci were found in the marrow in 38 of the 62 sections examined (61.2 per cent). It is to be remembered that this indicates not the incidence of lesions in these particular bones but the incidence of lesions in single microscopic sections from these bones. Surely the examination of more sections or of larger areas from each bone would have increased the incidence.

Lymphogranulomatous foci were found in one or more sections in 11 of the 14 cases examined (78.6 per cent). In 3 cases every section examined showed lesions. In each of 3 cases which showed no involvement, only three different bones were examined. A greater sampling of the skeleton might well have disclosed lesions in these cases. The different bones showed approximately the same incidence of involvement if those are disregarded which were sampled in only a few cases. It is

TABLE 4.—*Distribution and Incidence of Occult Lymphogranulomatous Foci on Random Sampling of the Marrow in Cases of Hodgkin's Disease*

	Bone								
Identification No.	Rib	Sternum	Lumbar Vertebral Body	Dorsal Vertebral Body	Femur	Clavicle	Metatarsal	Ilium	Frontal Bone
3309	0	..	..	+	0	+	..	..	..
3422	0	..	0	..	0	..	..	..	..
3603	0	..	+	..	0	..	..	..	..
4234	0	+	+	..	..	..	..	..	..
4255	+	+	+	+	+	..	..	..	..
4375	0	0	..	..	0	..	..	..	..
4442	+	0	+	..	+	..	0	..	..
4596	+	+	+	+	+	..	0	..	..
4626	+	+	0	+	..	..	..	..	..
4832	0	0	0	..	..	..	..	..	..
4926	+	+	+	+	+	0	0	+	0
5137	+	0	+	0	+	..	..	0	..
5280	+	+	+	+	..	..	..	+	..
5591	+	+	+	..	..	..	..	..	..
Percentage positive	57.1	63.7	74.9	85.8	55.5	50.0	0.0	66.6	0.0

+ indicates granulomatous foci; 0 indicates no granulomatous foci.

possible, however, that a larger series of cases would reveal differences in distribution which are not evident here.

These data serve to demonstrate the common and the widespread involvement of the marrow. They also show that the distribution of the lesions in the marrow is quite different from that of the clinically significant osseous lesions shown in table 3.

SIGNIFICANCE OF THE LYMPHOGRANULOMATOUS LESIONS IN THE MARROW

Pain.—One of the commonest and most distressing symptoms in Hodgkin's disease is pain. It may be fleeting, persistent or migratory and may be located in the back, the extremities, the head and elsewhere. It is often attributed to the pressure of enlarged lymph node masses on nerves, to invasion of nerves or to involvement of viscera, even though such lesions are not demonstrable. Some of it is undoubtedly pain of bones. Many authors have mentioned the high incidence of pain as a symptom in series of cases, and some of them have recognized it as pain of bones.[31]

31. (*a*) Craver, L. F., and Copeland, M. M.: Arch. Surg. **28**:1062, 1934. (*b*) Goldman, L. B.: J. A. M. A. **114**:1611, 1940. (*c*) Krueger, F. J., and Meyer, O. O.: J. Lab. & Clin. Med. **21**:682, 1936. (*d*) Cunningham, W. F.: Am. J. M. Sc. **150**:868, 1915. (*e*) Morrison.[23a]

Since widespread involvement of the marrow has been demonstrated in this study to be the usual condition, it is possible that this explains much of the pain which has previously been obscure. The remittent character of some of the pain might well be explained on the basis of successful destruction of the bone at the site of the lesion or by retrogressive changes in the lymphogranulomatous lesion itself.

Pain may be the first symptom, and it may be present before lymph nodes are appreciably enlarged.[31b] On the other hand, some lesions of bone large enough to be revealed by roentgenograms do not produce pain at all times.[31a]

Anemia.—The anemia which occurs so commonly in Hodgkin's disease has not been fully explained. In view of the prevalence of lesions in the marrow the possibility that it is due to the replacement of hemopoietic marrow must be considered.

The anemia can hardly be explained on this basis except in the rare and unusually extensive involvement, such as that observed by Herscher and Stein.[21b] In most instances the lesions are nodular and disseminated, at least in the majority of bones, so that much marrow persists between them.

Marchal and co-workers[32] described erythrophagia referable to the macrophages and attributed the anemia to it. Piechl [33] and Samek and Archi [34] have described cases in which the anemia resembled an aplastic type. These appear to have been unusual cases, and they do not explain the anemia usually encountered.

Sternal Puncture.—The question arises as to the value of sternal puncture in the diagnosis of Hodgkin's disease, especially in view of the high incidence of involvement of the sternum (7 of 11 cases). On theoretic grounds the procedure would not be expected to be reliable for three reasons: First, not every sternum showed the specific lesions. Second, the lesions usually occupied a small proportion of the marrow space as compared with the marrow itself, and the needle, on the basis of chance, would probably aspirate the latter. Third, the specific involved marrow was firmer than the noninvolved marrow. It tended to have much fibrous tissue, which bound it together, making aspiration difficult. It is generally agreed that the marrow itself shows no picture diagnostic of Hodgkin's disease, so that the specific tissue must be obtained if the diagnosis is to be made by this method.

The value of sternal puncture as reported in the literature has been in accord with the foregoing comment. Barasciutti [35] and Paraf and co-workers [161] have reported diagnostic failures. Váradi [36] had one positive result from sternal puncture, Sternberg cells appearing in the aspirate. He admitted, however, that his result was exceptional.

It is probable that surgical biopsy of the sternum would be more reliable than aspiration biopsy.

HODGKIN'S DISEASE AS A DISORDER OF THE RETICULOENDOTHELIUM

The incidence and the distribution of specific lesions reported in the present study may contribute to an understanding of the essential nature of this disease. Hodgkin's lymphogranulomatosis is often classified with the diseases of the lymphatic system. It should perhaps more appropriately be regarded as a disease of the reticuloendothelial system on the basis of its distribution in the body and the type of cell which is abnormal.

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32. Marchal, G.; Mallet, L.; Fressinaud, L., and Brun, C.: Bull. et mém. Soc. méd. d. hôp. de Paris **59**:21, 1941.
33. Piechl, N.: Wien. Arch. f. inn. Med. **34**:337, 1941.
34. Samek, E., and Archi, A.: Haematologica **15**:645, 1934.
35. Barasciutti, A.: Diag. e tec. di lab. **8**:481, 1937.
36. Váradi, S.: Sang **12**:106, 1938.

Two problems of a fundamental nature arise in this connection. The first is the problem of whether the disease is essentially a neoplasm or an infectious granuloma. Despite the insistence of Uehlinger [13] that the osseous lesions point to the latter, it must be conceded that they offer no conclusive evidence for either theory.

The second problem is concerned with the essential nature of the cells undergoing the principal proliferation. Except for Medlar,[30] who regarded the disease as megakaryocytoblastoma, investigators have held either that it is a disease of the reticuloendothelium or that it is a disease of the lymphatic series of cells. Although both Sternberg [2] and Reed [4] recognized the endothelial nature of the large cells which characterize this disease, controversy has persisted regarding the nature of the smaller, more numerous "Hodgkin's" [37] cells. Those who have expressed the opinion that Hodgkin's disease is a type of lymphoblastoma include Mallory,[38] Tsunoda,[39] Dietrich,[40] Warthin,[41] Wellbrock and Loughery [42] and others. On the other hand, Piney,[43] Favre and Croizat,[44] Pullinger,[45] Robb-Smith,[46] Potter,[37] McJunkin [47] and others have expressed the belief that this is a type of reticuloendothelioma or a disease of some cell precursor thereof. Because of the lability and the close histogenetic relationship of the cells of the hemopoietic system, perhaps the problem cannot be solved by a cytologic approach.

A reevaluation of the distribution of the lymphogranulomatous lesions in the body offers some statistical data which might be admitted as evidence. Is the distribution of the lesions more nearly that of the lymphatic than that of the reticuloendothelial system?

Morphologically and functionally a number of organs and tissues belong to both of these systems. Obviously, the incidence of their involvement cannot be used as evidence in favor of either system. Of organs which are dual in this respect the outstanding examples are the lymph nodes and the spleen. When examined microscopically they show the specific lesions in nearly 100 per cent of cases.

While there are no lymphoid tissues in the body which are entirely devoid of reticuloendothelial cells, the lymphoid tissues of the ileum and colon, the thymus and the tonsils and other lymphoid tissues of the nasopharynx approach lymphoid purity most nearly. They are involved in lymphogranulomatosis occasionally, but probably in not over 10 per cent of cases, which is roughly the relative proportion of reticuloendothelial cells and lymphocytes in them.

On the other hand, while there are tissues such as the liver and the marrow which are rich in reticuloendothelial cells, they are not without lymphocytes in small numbers, although they normally contain no lymphoid follicles. These tissues are commonly the site of lymphogranulomatous lesions. The liver is involved in

37. Potter, E.: Arch. Path. **19**:139, 1935.
38. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1929.
39. Tsunoda, T.: Virchows Arch. f. path. Anat. **204**:265, 1911.
40. Dietrich, A.: Folia haemat. **13**:43, 1912.
41. Warthin, A. S.: Ann. Surg. **93**:153, 1931.
42. Wellbrock, W. L. A., and Loughery, H. B.: Am. J. Clin. Path. **1**:455, 1931.
43. Piney, A.: Arch. Path. **2**:301, 1926.
44. Favre, M., and Croizat, P.: Ann. d'anat. path. **8**:838, 1931.
45. Pullinger, B. D., in Horder, T., and others: Rose Research on Lymphadenoma, Bristol, John Wright & Sons, Ltd., 1932.
46. Robb-Smith, A. H. T.: J. Path. & Bact. **47**:457, 1938.
47. McJunkin, F. A.: Arch. Path. **2**:815, 1926.

about 60 per cent of cases (Wallhauser) and the marrow in about 70 per cent (if not actually 100 per cent), as was shown in table 4.

On the basis of these statistics it appears that the distribution of lymphogranulomatous lesions is more closely related to the reticuloendothelial than to the lymphatic system, and this may constitute evidence that it is a disease of the former.

On the other hand, in lymphosarcoma of the lymphocytic type the distribution of the lesions corresponds more nearly to that of the lymphatic than to that of the reticuloendothelial system, as is to be expected. This statement appears to hold despite the fact that the lymphocytic and the reticulum cell type of lymphosarcoma are often not sharply separated in the literature. Vieta and co-workers [25] found that skeletal lesions were about twice as common in cases of lymphogranuloma as in cases of lymphosarcoma as judged by both clinical examination and necropsy. The liver is frequently spared in the lymphocytic type of lymphosarcoma. On the other hand, the great majority of tumors in the thymus are diagnosed as lymphosarcoma.[48] Tonsillar and gastrointestinal involvement in lymphosarcoma are relatively common, according to Ewing.[29]

SUMMARY

An attempt to find the absolute incidence of the lymphogranulomatous involvement of the marrow in cases of Hodgkin's disease by microscopic examination of small samples of various bones selected at random showed lymphogranulomatous foci in one or more sections in 11 of 14 consecutive cases of Hodgkin's disease (78.6 per cent). The lymphogranulomatous foci were found in 38 of the 62 sections examined. It is believed that practically every case would show lesions of the marrow if enough bones could be examined thoroughly.

It is suggested that these widespread and obscure lesions may be responsible for the pain which patients with Hodgkin's disease often experience. They do not explain the anemia on a replacement basis in the average case. Their nature is such that aspiration of marrow for diagnostic purposes is likely to result in failure.

The distribution of the lesions in Hodgkin's disease, unlike that in lymphosarcoma, resembles more closely the distribution of the reticuloendothelial than that of the lymphatic system. This observation may constitute additional evidence that Hodgkin's disease should be regarded as a disorder of the reticuloendothelium.

48. Crosby, E. H.: Am. J. Cancer **16**:461, 1932. Ewing.[29]

Notes and News

Awards.—Tom D. Spies has been presented with the occasional research medal of the Southern Medical Association "in recognition of his outstanding contributions to our knowledge of the science of human nutrition, especially in his elucidation of earlier and better methods of diagnosis and treatment of disease."

Richard E. Shope, Princeton, N. J., a member of the Rockefeller Institute for Medical Research, has been awarded the John Scott Medal and "premium" of $1,000 by the city of Philadelphia for his "discovery of the complex etiology of swine influenza."

At the celebration on October 27 of the centennial of the Western Reserve University School of Medicine the doctorate in science was conferred on George H. Whipple, University of Rochester, and on Reginald Fitz, Boston.

Society News.—The Federation of American Societies for Experimental Biology has decided to omit the 1944 meeting for the same reasons that the 1943 meeting was omitted. The next meeting will be held in 1945 in Cleveland.

Appointment.—William Dock, since 1940 professor of pathology at Cornell University Medical College, New York, has been appointed professor and chairman of the department of medicine at the University of Southern California School of Medicine, Los Angeles.

Charles L. Mayer Award.—Manuscripts and published articles submitted for the 1943 Charles L. Mayer Prize are being received by the National Science Fund of the National Academy of Sciences, 515 Madison Avenue, New York 22. The closing date for the receipt of contributions is Jan. 15, 1944.

The award will be made for an outstanding contribution to present day knowledge of factors affecting the growth of animal cells with particular reference to human cancer, and the advisory committee assisting the National Science Fund in administering the award is prepared to consider contributions published during 1943 or in manuscript. In addition, the committee requests recommendations from scientists or persons whose present work comes within the field for which the award is offered and who are achieving outstanding results.

Gifts to the Royal College of Surgeons, England.—An immediate gift of £100,000 for the endowment of the department of pathology and the institution of a chair of human and comparative pathology to the Royal College of Surgeons of England by W. H. Collins, of Buckinghamshire, is reported. Mr. Collins has informed the college that he has made provision in his will for a bequest of a further £100,000 for the endowment of the department of anatomy and the institution of a chair of human and comparative anatomy. He wrote:

"I have been greatly impressed with the value of the departments of anatomy and pathology, which have made the Royal College of Surgeons of England famous all over the world. I have seen what grievous injury your departments have suffered as the result of enemy action, and appreciate what a gigantic task it will be to restore them to their unique position in the scientific world. To embark upon this task it is essential that the departments shall have an assured income from endowments. . . . I trust that my gifts will enable the council to proceed with confidence with their responsible task and to engage the services of men of outstanding ability to assist them in their labors."

Books Received

REACTION TO INJURY. PATHOLOGY FOR STUDENTS OF DISEASE BASED ON THE FUNCTIONAL AND MORPHOLOGICAL RESPONSES OF TISSUES TO INJURIOUS AGENTS. Wiley D. Forbus, M.D., professor of pathology, Duke University, and pathologist to the Duke Hospital. Pp. 797, with 532 illustrations, including 20 kodachromes reproduced in color. Price $9. Baltimore: Williams & Wilkins Company, 1943.

This book is based on the conception that the fundamental element in disease is the reactions of the patient to environmental factors—reactions of resistance, of submission, of adaptation. Disease is the expression of one or more of these reactions. "All three forms of reaction to injury may become operative in the course of development of a single disease entity. A disease entity must be grouped therefore according to its primary or dominant reaction." The general plan of the book at once reminds one of MacCallum's Textbook of Pathology, and in the preface the author acknowledges his deep obligation to Dr. MacCallum for guidance and encouragement.

The volume at hand deals with the nature and the causation of disease and with the reactions of resistance, i. e., the inflammatory process and the infectious diseases. The parts dealing with the submissive and adaptive types of reaction are to be published later.

The introduction contains instructive chapters on pathology, the science of disease; the nature of disease; the extrinsic inanimate and animate factors in the production of disease. Then comes a chapter on the nature and significance of inflammation, followed by sections on reactions to injury by bacteria through acute and chronic inflammation and antibody formation (inflammatory diseases of bacterial origin); on reaction to injury by obligate cellular parasites through inflammation and related mechanisms, i. e., the diseases caused by viruses and rickettsias; and on reaction to injury by foreign bodies, treponemes and fungi through primary inflammatory alteration of the reticuloendothelial system (chronic granulomatous diseases). In these sections (626 pages) the nature, the etiology and the structural changes of the principal infectious diseases receive thorough consideration. The clinical and the morphologic evolution of the diseases is notably well described.

A special feature of the volume is the illustrations, 90 per cent of which are original. The illustrations—gross, microscopic and kodachrome—and the legends maintain a uniformly superior standard of usefulness throughout. At the ends of the chapters are lists of writings which have been found to be of special value. The book "has been in the making" for several years. The style is lucid; the treatment, scholarly and comprehensive. The student of this book gains a broad conception of the central problems and relationships of diseases. The volume is an outstanding contribution to the textbook literature of pathology, and the publication of the coming parts will be awaited with much interest.

At the end of his discussion (page 27) of the development of the science of disease the author writes: "Present-day investigation proceeds along a few well defined lines. Bacteriology and its daughter science, immunology, are still beset with interesting and important problems, especially in the outer zones where it seems that the questions relating to the various filtrable viruses belong. The more highly organized microscopic organisms, the fungi, related closely to the bacteria, are rich and promising materials for study from the point of view of the etiology of disease. The application of the principles of biological chemistry to the study of complicated processes such as those involved in nutrition and the investigation of the general control of metabolism through hormonal mechanisms seem now to be the most promising fields of work. Pathological anatomy as a base line from which the science of medicine grows surely will be continuously and thoroughly cultivated even though its major contribution to the development of our knowledge of the nature of disease has been made. It seems not unlikely, however, that we may be permitted soon to see the ultramicroscopic structural changes that we have a right to believe from past experiences must accompany physico-chemical intracellular activity. If and when this becomes possible, we shall enter a new world of highly complex structures, comparable in many respects to that disclosed to us when the cell was first seen just a little more than a hundred years ago. The development of a new and inconceivably complex pathological anatomy must of necessity follow such an evolution since not cells but chemical compounds and even electrically active, elementary bodies doubtless will be found to play the vital roles in this ultra-microscopic world."

WHITE BLOOD CELL DIFFERENTIAL TABLES. Theodore R. Waugh, M.D., C.M., pathologist in chief, Royal Victoria Hospital; associate professor of pathology, McGill University; con-

sulting pathologist, Montreal Homeopathic Hospital, Montreal, Quebec. Pp. 130. Flexible cloth. Price $1.60. New York and London: D. Appleton-Century Company, 1943.

Tables are given for computing rapidly the actual number per cubic millimeter of the white blood cells of each type from the differential percentages.

IRRIGACIÓN NORMAL DEL NÓDULO DE KEITH Y FLACK, TAWARA, HAZ DE HIS Y SUS RAMAS. ESTUDIO PREVIO DE LA DISTRIBUCIÓN DE LOS GRUESOS VASOS CORONARIOS CARDIACOS. Eduardo F. Lascano. Pp. 100, with 25 illustrations. Buenos Aires: Biblioteca, Facultad de ciencias médicas, 1942.

This is an illustrated monograph on the results of an anatomic investigation of the blood supply of the sinoauricular node, Tawara's node, the bundle of His and its branches.

ORTHOPEDIC NURSING. Robert V. Funsten, M.D., professor of orthopedic surgery, University of Virginia Medical School and University of Virginia Hospital School of Nursing, Charlottesville, Va., and Carmelita Calderwood, R.N., A.B., consultant in orthopedic nursing, National League of Nursing Education, New York. Pp. 602, with 181 illustrations. Price $3.75. St. Louis: The C. V. Mosby Company, 1943.

CLINICAL DIAGNOSIS BY LABORATORY EXAMINATIONS. John A. Kolmer, M.D., D.P.H., D.Sc., LL.D., L.H.D., professor of medicine in the Temple University School of Medicine and School of Dentistry; director of the Research Institute of Cutaneous Medicine, Philadelphia. Pp. 1239, with 75 illustrations. Price $8. New York and London: D. Appleton-Century Company, 1943.

CLINICAL LABORATORY METHODS AND DIAGNOSIS. A TEXTBOOK ON LABORATORY PROCEDURES WITH THEIR INTERPRETATION. R. B. H. Gradwohl, M.D., D.Sc., director of the Gradwohl Laboratories and Gradwohl School of Laboratory Technique, St. Louis. Volumes I and II. Third edition. Pp. 2130, with 783 illustrations; index pp. 100. Price $20. St. Louis: C. V. Mosby Company, 1943.

MEMORIAL HOSPITAL FOR THE TREATMENT OF CANCER AND ALLIED DISEASES: TRIENNIAL REPORT, 1940-1941-1942. Pp. 144. New York: 444 East 68th Street, 1943.

TIFUS EXANTEMÁTICO: ETIOLOGIA—CLINICA—PROFILAXIS. Prof. Dr. G. Clavero and Dr. F. Perez Gallardo. Pp. 166, with 41 illustrations. Price 25 pesetas. Madrid: Gráficas Afrodisio Aguado, 1941.

CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS: II. ENVIRONMENTAL STUDIES. A.—Report by the Committee on Industrial Pulmonary Disease. B.—Reports on Physical, Chemical and Petrological Studies. Medical Research Council Special Report Series no. 244. Pp. 222, illustrated. Price 10s 6d. London: His Majesty's Stationery Office, 1943. New York: British Information Services, 1943.

THE FIFTY-EIGHTH ANNUAL MEDICAL REPORT OF THE TRUDEAU SANATORIUM AND THE THIRTY-EIGHTH SUPPLEMENT FOR THE YEAR ENDING SEPTEMBER 30, 1942, TOGETHER WITH THE TWENTY-SIXTH COLLECTION OF THE STUDIES OF THE EDWARD L. TRUDEAU FOUNDATION FOR RESEARCH AND TEACHING IN TUBERCULOSIS. Saranac Lake: Edward L. Trudeau Foundation, 1943.

ANNUAL REPORT OF THE SARANAC LABORATORY FOR THE STUDY OF TUBERCULOSIS OF THE EDWARD L. TRUDEAU FOUNDATION FOR THE YEAR 1942. Pp. 32. Saranac Lake: Edward L. Trudeau Foundation, 1943.

CONTRIBUTIONS TO THE STUDY OF TUBERCULOSIS. By the Research Department of the National Jewish Hospital at Denver. Volume XV, 1940-1942. Various pagination. Denver: National Jewish Hospital, 1943.

FORTIETH ANNUAL REPORT, 1942-1943, OF THE IMPERIAL CANCER RESEARCH FUND, APRIL 1943. Pp. 31. London: Royal College of Surgeons, 1943.

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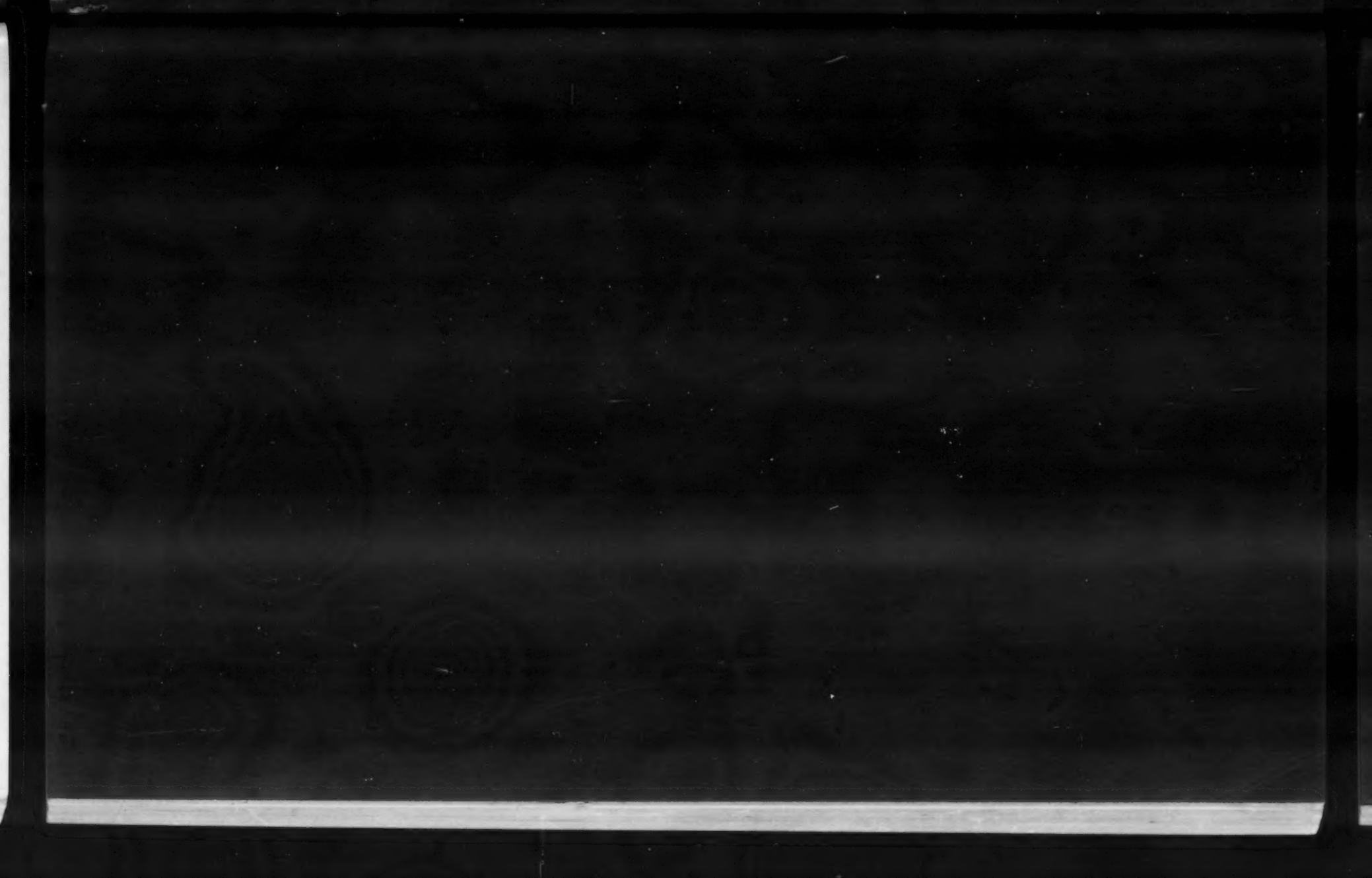

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